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THE RELATION OF ENVIRONMENTAL CONDITIONS TO THE PHENOMENON OF PERMANENT WILTING IN PLANTS.

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Alabama Polytechnic Institute.

ABSTRACT.[1]

The experiments described in this paper had as their chief purpose to discover (a) whether the water content of a given soil at the time when plants growing therein become permanently wilted remains a constant when wilting occurs under widely varying environmental conditions, and can therefore be determined with accuracy by calculation from the known physical constants of the soil, and (b) whether there is a definite and constant relation between the water content of the plant and that of the soil at the time of wilting, under such varying conditions. The work was performed at the Desert Laboratory of the Carnegie Institution, at Tucson, Arizona, during the summer of 1911. The plants used were *Zea mays* L., *Phaseolus vulgaris* L., *Xanthium commune* Britton, *Martynia louisiana* Mill., and *Physalis angulata* var. *Linkiana* Gray.

The chief results follow:

1. For large numbers of turgid individuals of the same age and species, the average water content of all the normal functioning leaves is approximately a constant under the midsummer conditions of southern Arizona. Departures of individual plants from the average normal foliar water content for the species were few and small in the five species studied.

2. Plants beginning to lose turgor in the youngest leaves show an average foliar water content which is fairly constant for the species and which bears a definite ratio to the average normal water content for turgid individuals of that species.

3. Temporary wilting of the younger foliage is of frequent occurrence and is conditional only upon a temporary, but more or less prolonged, maintenance of the rate of transpiration at a magnitude in excess of the rate of absorption for the same period; hence, this form of wilting is not related merely to the moisture content of the soil, but also to aerial conditions, especially to the evaporating power of the air and to the intensity of solar insolation.

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[1] The manuscript of this paper was received March 25, 1913. This abstract was preprinted without change, from these types and was issued as Physiological Researches Preliminary Abstracts vol. 1, no. 1, July, 1913

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4. The moisture content of the normal functioning leaves, taken collectively, at the time of *permanent* wilting, was found to be approximately constant for all individuals of the same species, at the same stage of development. This constant is unaffected by the external conditions under which wilting occurs; wiltings under all the conditions of the experiments yielded practically identical results.

5. When wilting occurs in a shaded moist chamber having a nearly saturated atmosphere, the ratio of soil moisture content at permanent wilting, as found by actual determination, to that obtained by calculation from the physical constants of the soil, is approximately unity for all the soils used.

6. For wiltings occurring under partial shelter or in the open, with relatively high evaporation rates, the ratio of observed to calculated soil moisture content at permanent wilting is always greater than unity.

7. Under any given set of aerial conditions the observed soil moisture content at permanent wilting is approximately a constant for each of the soils used, and its value increases with increase in the rate of transpiration, being greater under conditions of high evaporation intensity and declining with decrease in the evaporating power of the air. For a series of plants grown in any one soil, and wilted under a number of different aerial conditions, all with relatively high evaporation rates, as many different soil moisture contents at permanent wilting are obtained as there are sets of conditions. Each of these is approximately a constant for all plants of that species undergoing wilting under identical conditions.

8. The departure of the observed from the calculated soil moisture content at permanent wilting is greatest in soils having very low saturation capacities, and becomes steadily less as the water-holding power of the soil increases.

9. Under the conditions of maximum evaporation prevailing at Tucson in the summer, the departure of observed from calculated soil moisture contents at permanent wilting is extremely marked for all soils; permanent wilting in the open occurs with a soil moisture content from 30 to 40 per cent. in excess of that present when the same or similar plants are wilted in a moist chamber.

10. The progressive rise in the evaporating power of the air, as it occurs in the open during the progress of a single day, affects the moisture residue in the soil at permanent wilting as observed for plants wilting on that day; those plants attaining the permanently wilted condition at or near noon invariably show higher soil moisture percentages than do those wilting at an earlier hour.

11. Transfer of plants from a location of lower to one of higher evaporation rate, or the reverse, just prior to the onset of permanent wilting, does

not alter these general results; the soil moistures found are approximately similar to those obtained for plants grown and wilted under identical conditions in the same situation.

12. Permanent wilting is a definite protoplasmic condition, brought about by reduction of the water content to such a point that loss of turgor has become established throughout the entire plant. When such a loss of turgor has been rapidly produced, as by high transpiration in the open at Tucson, the root hairs are found to be plasmolyzed, but such root hairs promptly resume their normal appearance and functions when water is again applied to the soil. On the other hand, when loss of turgor has been very slowly attained, as is the case under conditions of high relative humidity and but slight air movement, there results a general death of the root hairs. This is shown by more pronounced plasmolysis than that occurring in the case just mentioned, by cessation of protoplasmic streaming, and by subsequent disintegration, even after abundant water has been supplied. Recovery of turgor in such plants, because of the great reduction of functional absorbing surface, is a slow process requiring from 48 to 72 hours for its completion, whereas plants wilting in the open with high transpiration rates recover about twice as rapidly after water has been supplied to the soil.

13. The condition of permanent wilting may result from the operation of either one or both of two factors, (a) decrease in the soil moisture content and (b) loss of water from the plant by transpiration. The attainment of the permanently wilted condition indicates a certain degree of reduction of the amount of water contained within the organism. When this condition is reached under environmental conditions such as those of a moist chamber or an adequately controlled greenhouse,—with low evaporation rates and without great variation in the evaporating power of the air,—it also indicates a concomitant reduction of the amount of soil water to a definite limit. Marked increase in the evaporating power of the air accelerates the outgo of water without producing a proportionate increase in its rate of entrance from the soil. With every increase in transpiration rate above a certain limit, this rate becomes, therefore, more and more significant as a factor determining the extent to which the soil water may be exhausted by the plant before the advent of permanent wilting. Thus, permanent wilting under high rates of evaporation does not at all indicate that the available soil moisture has been exhausted. Instead, it merely indicates the reduction of the soil moisture content to a magnitude which corresponds to the residue of water left in the soil at the time when excess of transpiration over absorption has brought the entire plant into the permanently wilted condition. Repeated determinations, under widely varying conditions but with relatively high evaporation rates, show that the magnitude of this

residue is directly related to the intensity of the evaporating power of the air.

14. Excepting with relatively low rates of transpiration, any method which employs the permanent wilting of the plant as an indicator of soil properties must be accompanied by adequate characterization of the aerial conditions under which the tests were carried out; otherwise the results cannot be available for comparison with those obtained under other aerial conditions.

INTRODUCTION.

The belief that different species of plants show very considerable differences in their ability to withdraw water from a given soil, while more or less explicitly stated in many current textbooks of plant physiology and ecology, and everywhere held by practical agriculturists, rests upon a basis of casual observation rather than upon any considerable body of carefully controlled experimentation. Possibly no other problem of equal practical importance has so completely escaped attention at the hands of plant physiologists. The studies found in the older literature were, for the most part, made before increasing knowledge of soil physics rendered possible any accurate definition of the soils used, and there is a general absence of such data as would permit accurate or detailed comparison of the reported results.

Notwithstanding the fragmentary nature of the data given, the earlier literature is in quite general agreement in showing that plants of differing species grown in a given soil show marked and unmistakable differences in the soil moisture content present when cessation of growth occurs and wilting begins. Heinrich,[2] using a large number of field crops, determined the percentage of water present in the soil when wilting began, finding very marked differences in these percentages for any given soil; for example, the percentage of water present in one calcareous soil ranged from 5.07 per cent. for potatoes to 11.30 per cent. for horse bean, at the time when wilting began. His conclusion that cultivated crops show specific differences in their power to withdraw water from the soil has been questioned by Cameron and Gallagher,[3] who have deduced from Heinrich's results the conclusion that "the moisture content at which wilting of plants begins is approximately constant for any one soil" ([08], page 59), but the differences shown by Heinrich's figures are scarcely such as to warrant this generalization. Edmond Gain[4] found very considerable and fairly constant specific differences for three species when wilted in five different soils. Hedgcock,[5] who has made the only extensive series of experiments with a considerable number of plants under differing sets of measured conditions as regards light, humidity, temperature and soil moisture, found that the ability of plants to withdraw water from the soil varied widely, being least for hydrophytes and progressively increasing in magnitude throughout a series terminating with extreme xerophytes. When grown in the same soil under identical aerial conditions, the percentages of soil moisture present at the beginning of wilting were from 3 to 3.07 per cent. for pronounced xerophytes, while for mesophytes in the same soil the range was from 5 to 12 per cent. Well marked generic and specific differences in capacity to withdraw water from the soil were shown by his plants, and variations in the environmental conditions materially modified this capacity. Livingston[6] concluded from a comparison of the soil moistures found at wilting for such xerophytes as *Allionia, Boerhavia*, and *Euphorbia* with those for the mesophytes *Phaseolus, Helianthus* and *Vicia*, that "desert forms show an adaptation to existence in dry soil, being able to exist in soil somewhat drier than those needed by plants of a humid region, but this adaptation is comparatively slight and cannot be considered of prime importance" ([06], pages 65-69). Brown[7] has found that the moisture content of the soil at the time at which wilting begins is directly dependent, as to its amount, upon the evaporating power of the air at the time of wilting. This author emphasizes the fact that determinations of soil moisture at the time when wilting begins can have little comparative value except as they are accompanied by quantitative measurements of the accompanying atmospheric conditions.

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[2] Heinrich, R., Zweiter Bericht über die Verhältnisse und Wirksamkeit der landwirtschaftlichen Versuchsstation zu Rostock. 1894.

[3] Cameron, F.K. and Gallagher, F.E., Moisture content and physical condition of soils. U.S. Dept. Agric. Bur. Soils Bull. 50. 1908.

[4] Gain, Edmond, Action de l'eau du sol sur la vegetation. Revue Gen. Bot., 7: 15-26, 71-84, 123-[illegible]

Briggs and Shantz,[8] in a series of papers dealing with the problem primarily from the point of view of soil physics rather than that of plant physiology, have announced conclusions which stand in sharp contrast to those of earlier workers. Working with about twenty soils which differed widely in water-holding capacity and other physical characters, and employing more than one hundred species and varieties of plants ranging from typical hydrophytes to extreme xerophytes such as the cacti, these investigators found that the moisture content of any particular soil, at the time when plants rooted therein had become strongly wilted, was practically a constant. The authors conclude that this " wilting coefficient " for a given soil is a fixed quantity for all species grown in it, at all stages of their development, and that differences in environmental conditions during development and wilting are without effect upon its magnitude. Furthermore, they found the relations between "wilting coefficient," hygroscopic and saturation coefficients, moisture equivalent, and the data of the mechanical analysis to be such that, when any one of these is known for a given soil, the others may be computed by means of mathematical formulae. These results have recently been employed also by Kearney.[9]

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[5] Hedgcock, George G., The relation of the water content of the soil to certain plants, principally mesophytes. Bot. Surv. Nebraska, **6**: 5-79. 1902.

[6] Livingston, Burton E., The relation of desert plants to soil moisture and to evaporation. Carnegie Institution Wash. Publ. 50. Washington. 1906.

[7] Brown, Wm. H., The relation of evaporation to the water content of the soil at the time of wilting. Plant World, **15**: 121-134. 1912.

[8] Briggs, Lyman J. and Shantz, H.L., A wax seal method for determining the lower limit of available soil moisture. Bot. Gaz. **51**: 210-219. 1911.

——— Application of wilting coefficient determinations in agronomic investigations, Proc. Am. Soc. Agron. **3**: 250-260. Washington, 1912.

——— The wilting coefficient and its indirect determination. Bot. Gaz. **53**: 20-37. 1912.

——— The relative wilting coefficients for different plants. Bot. Gaz. **53**: 229-235. 1912.

——— The wilting coefficients for different plants and its indirect determination. U.S. Dept. Agric. Bur. Plant Ind. Bull. 230. 1912.

——— Die relativen Welkungskoefizienten verschiedener Pflanzen. Flora **105**: 224-240. 1913.

It is unfortunate that the term "wilting coefficient" should have been chosen to designate a condition of the soil with respect to its contained water, in as much as the term would by definition logically apply to that which wilts, namely the plant itself, and is apt to be understood as applying to the water content of the plant, when used without definition. To avoid confusion, one must employ the phrases "wilting coefficient of the soil" and "wilting coefficient of the plant." Since the first of these terms is in reality a misnomer, it has seemed advisable to avoid their use entirely and to employ the phrases "soil moisture residue at time of permanent wilting" and "leaf moisture content at time of permanent wilting," which, while somewhat cumbrous, have the merit of freedom from ambiguity.

The conditions and the methods of experimentation employed by Briggs and Shantz differ materially in a number of particulars from those of earlier workers. The "wilting coefficient" adopted for their work is the percentage of water present in the soil when wilting has proceeded so far that the plant cannot recover in an approximately saturated atmosphere, without previous addition of water to the soil. This residue is therefore not to be compared with the quantities of soil moisture found at the time when loss of turgor in the younger leaves—the condition usually termed "wilting" in physiological literature—first becomes apparent. Brown ([12], page 126) produced three or four of these temporary wiltings with subsequent recovery, without addition of water, by transferring his plants to successively less and less severe conditions of evaporation, and a similar phenomenon may be observed in the open during any period of hot, dry weather. The condition which has just been termed *temporary wilting* may or may not pass over into the more serious condition of *permanent* wilting, from which recovery is impossible, even in a saturated atmosphere, without addition of water to the soil. Permanent wilting was employed by Briggs and Shantz as the criterion for the determination of their "wilting coefficients," while the wilting with which Brown's studies deal was obviously not permanent wilting, but temporary loss of turgor in the younger parts. This is also the case with the wiltings studied by Heinrich [94], by Gain [95], by Hedgcock [02], and by Livingston [06].

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[9] Kearney, T. H., The wilting coefficient for plants in alkali soils. U. S. Dept. Agric. Circular 109 (pages 1-25), 1913.

The experiments of Briggs and Shantz [12, *3,4*] were conducted in a greenhouse with an average temperature of 70° C. and an average relative humidity of 85 per cent.; changes in humidity and air temperature were gradual and slight, while the temperature of the soil was kept approximately constant by a constant current of water around the pots. Heinrich [94] and Gain [95] are silent as to the conditions of their experiments; Hedgcock [02] and Brown [12] worked under a variety of atmospheric conditions which are described in some detail; Livingston [06] gives records of temperature, humidity, and evaporating power of the air. In the determinations of soil moisture recorded in the older literature there has been no uniformity either in the use of means to prevent drying of the soil at top and sides of the containers, in the size of the pots used, in the age of the experimental plants, or in the degree to which the soil mass had been occupied by the roots prior to the beginning of the experiments. The methods employed for determining the saturation capacities and hygroscopic coefficients of the soils used are variable, and atmospheric conditions, if considered at all, are often described only in the most general terms. Comparisons of the results recorded in the literature are therefore quite impossible, excepting in the most vague and general manner, and the formation of definite conclusions upon the relations between plant and soil water must await the adoption of standard and uniform methods of study.

Our knowledge as to the amount of the average or normal water content of any given plant throughout the various stages of its development is very imperfect; most of the data found in the literature of this subject were obtained in connection with studies dealing with the relative efficiency of various protective structures in reducing transpiration or in prolonging the period required for complete desiccation, and have therefore dealt for the most part with succulents and xerophytic forms. The papers of Fleischer[10] and of G. Schröder[11] give a number of such determinations, and the latter summarizes rather fully the older literature, especially as regards the extent of desiccation which may be endured by various succulents. Fleischer determined the normal water content of his forms and attempted to distinguish—by noting the amount of water present at the time when death of tissues began—between that portion of the contained water which may be regarded as stored in the tissues and that which is indispensable to the continuance of life. He used detached leaves or branches bearing several leaves. D. Schröder[12] made a somewhat extensive study of the course of withering in detached leaves of some thirty species, by determining the relative rates of water loss for stated periods, beginning with fully turgid leaves and ending with the attainment of constant air-dry weight. His tables give very full data as to the "relativer Wassergehalt" of full grown leaves of the species used, which included a number of mesophytes. The work of Cettli[13] upon the water balance in certain rock plants furnishes some data of like character. But in all these cases the authors have been concerned primarily with the extent of the resistance to drying displayed by individual adult leaves and with the structural features responsible therefor, rather than with the normal water content of the entire foliage. The figures given by Ebermayer[14] and by Konig[15] are largely derived from similar sources, and are in part based upon single determinations. The only extended study dealing directly with the average water content of large numbers of individuals of a species is that of Hedgcock [02], who made moisture determinations for a number of species and for both turgid and wilting plants, concurrently with studies of soil moisture content at time of wilting. He found that entire turgid plants of the same species had, at any given age, approximately the same water content regardless of differences in the soil or in the conditions under which they were grown. On the contrary, the water content of plants beginning to wilt varied with the soil, being always greater in clay, loess, and saline soils than in loam, humus, and sand.

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[10] Fleischer, E., Die Schutzeinrichtungen der Pflanzenblätter gegen Vertrocknung. Ber. Kais. Realgymnasium Dobeln, **16**. 1885. Abstract by Möbius, Bot. Centralb. **22**: 357-360. 1885.

[11] Schröder, G., Ueber die Austrocknungsfähigkeit der Pflanzen. Untersuchungen aus dem Bot. Inst. zu Tübingen, **2**: 1-52. 1886.

It has been assumed that for any plant there exists an optimum water content at which its development in any given stage may proceed most rapidly, and it has been suggested by Free[16] that this optimum water content for the plant may correspond more or less closely to that condition of the soil—its critical or optimum water content—at which it takes on its most favorable physical structure (a suggestion which can scarcely hold, since another factor, namely the rate of water loss from the plant through transpiration, must largely determine the favorable or unfavorable character of its water supply). While the optimum water content has been determined for a variety of soils,[17] the question whether there exists an actual optimum water content for the plant itself has not been investigated. Nor have we much information in regard to another matter of great practical and theoretical importance, namely, the extent of the variation in water content which growing plants may undergo without suffering temporary interruption of growth or permanent decrease of vitality.

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[12] Schröder, Dominicus, Ueber den Verlauf des Welkens und die Lebenszähigkeit der Laubblätter. Inaug. Diss. Univ. Gottingen 1909.

[13] Oettli, Max., Beitrage zur Oekologie der Fels-Flora. Untersuchungen aus dem Kurfurst- und Sentisgebiet. Zürich. 1905.

[14] Ebermayer, T., Physiologische Chemie der Pflanzen. Jena, 1882. Page 2.

[15] Konig, J., Chemie der Nährungs- und Genussmittel. Berlin, 1889. Theil 1, page 641.

[16] Free, E.E., Studies in Soil Physics, Plant World 14: 29-39, 59-66, 110-119, 164-176, 186-190. 1911 On this point, see pages 33-34 and 118-119.

[17] Cameron and Gallagher [08], pages 57-58.
Cameron, F.K., The Soil Solution. Easton, Pa. 1911. Page 24.

On *a priori* grounds it seems highly probable that there exist rather definite relationships between the water content of the plant and that of the soil which forms its source of supply. Just as there are fairly well defined limits above and below the "critical water content" within which the amount of soil water may vary without injury to the plant cover, so there must be for the plant a zone of safety, lying upon either side of its optimum water content, within which variations in the amount of water present are unaccompanied by injurious effects. Drying of the soil to a point below the critical water content must presently result, through a progressively decreased rate of absorption, in a corresponding decline in the water content of the plant toward the lower margin of the zone of safety here postulated. It is this plant condition, before actual wilting has occurred, which has been termed "incipient wilting" or "incipient drying" by Livingston[18] and Livingston and Brown.[19] The "Sättigungsdefizit" of Renner[20] appears to represent an early stage of this same phenomenon, as is pointed out by Livingston and Brown ([12], page 315), and by Brown ([12], page 122). This saturation deficit, concerning which Renner[21] has recently published further valuable observations, appears always to exist in plants where transpiration is in progress, even in the presence of most rapid growth; it should vanish only after all resistance in the water supply to peripheral cells has been for some time removed, as by prolonged maintenance of a water film over all the aerial as well as the subterranean surfaces. In this last case every cell and every membrane should have arrived at complete saturation, so that a water-absorbing force should no longer be exhibited at all, at the surfaces of either roots or leaves. With the inception of transpiration, this ideal condition could no longer hold, and saturation deficit must supervene, with its accelerating influence upon conduction and root absorption. (Renner [12, *1*].) With progressive increase in saturation deficit, the phenomenon dealt with by Livingston and Brown [12] should be encountered; their incipient drying appears to be Renner's saturation deficit in its more pronounced condition, when its retarding influence upon transpiration has become demonstrable, but still before actual wilting has occurred. Temporary and permanent wilting appear to be still more pronounced phases of a progressively increasing saturation deficit, and the further continuation of the drying process should eventually result in death and desiccation. If the transpiration of a plant which has reached the condition of incipient drying continue unchecked, the water content of the plant should steadily decline, with progressive decrease in the rate of absorption, until the lower limits of the zone of safety be crossed, when visible wilting begins. That there is a very considerable daily variation in the water content of the foliage when plants are subjected to very high evaporation rates, has been shown by Livingston and Brown [12] and also by Lloyd,[22] while rates of transpiration exceeding absorption have frequently been noted under such conditions (by Ebermayer [82], Free [11], Renner [11], and Eberdt[23]). It seems reasonable to assume that there exists, for the leaves of plants of a given species and developmental phase, rather definite quantities of water, each such quantity representing a definite stage of saturation of the tissues as a whole and a definite physical condition of the protoplasm. These constants might be: (1) a water content at saturation, at which the osmotic and imbibitional forces of the cells and their membranes are satisfied, a condition which must very rarely occur in nature; (2) an optimum water content, sufficient for maintaining growth and turgor despite moderate fluctuations resulting from lack of complete balance between absorption and outgo; (3) a wilting content, representing the amount of water present when turgor has just disappeared; and (4) a residue of moisture remaining when the plant has attained the permanently wilted condition. Should such more or less definite constants exist, they might be expected to hold under identical conditions for all members of a given species. They should also bear demonstrable relationships to the environment of the plant—the soil water supply, the physical characters of the soil, the evaporating power of the air—and should enable us to define the condition of a plant with respect to these relations as precisely as the statement of the critical moisture content appears to define the physical condition of the soil.

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[18] Livingston, Burton E., Light intensity and transpiration. Bot. Gaz. **52**: 417-438. 1911.

[19] Livingston, Burton E. and Brown, Wm. H., Relation of the daily march of transpiration to variation in the water content of foliage leaves. Bot. Gaz. **53**: 309-330. 1912.

[20] Renner, O., Experimentelle Beiträge zur Kenntniss der Wasserbewegung. Flora **103**: 171-247 1911.

[21] Renner, O., Versuche zur Mechanik der Wasserversorgung. 1. Der Druck in den Leitungsbahnnen von Freilandpflanzen. (Vorläufige Mitteilung) Ber. Deutsch. Bot. Ges. **30**: 576-641. 1912.

——— Versuche zur Mechanik der Wasserversorgung. 2. Ueber Wurzeltätigkeit. (Vorläufige Mitteilung) Ber. Deutsch. Bot. Ges. **30**: 642-648. 1912.

EXPERIMENTATION.

The experiments here described had as their purpose:

(1) To compare the calculated and observed soil moisture contents at permanent wilting of plants rooted therein, for a considerable number of soils having known physical constants, for several species of plants and under a variety of conditions of evaporation.

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[22] Lloyd, Francis E., Relation of transpiration to stomatal movement and water content of leaves in *Fouquieria splendens*. Plant World **15**: 1-14. 1912.

[23] Eberdt, O., quoted in Burgerstein, A., Die Transpiration der Pflanzen. Jena, 1904. Page 17.

(2) To make, concurrently with the above determinations, such a study of the water content of leaves of turgid and of wilting plants as might indicate
   (a) whether there is a correlation between water content of the soil and that of plant foliage at the time of temporary wilting;
   (b) whether such correlation, if present, is maintained under widely varying conditions of evaporation;
   (c) whether the water content of leaves at the time of permanent wilting is in any degree constant for the same species at a given stage of development and under a variety of aerial conditions; and
   (d) whether any definite relation exists between the normal water content of turgid leaves and their content at permanent wilting.

(3) To determine the effect upon these relations, should they be found to exist, of repeated wilting and recovery, under identical and under differing conditions.

The work was done at the Desert Laboratory, at Tucson, Arizona, under the auspices of the Department of Botanical Research of the Carnegie Institution of Washington, in the period from June to October, 1911. It is a pleasure to acknowledge my indebtedness to Professor Burton E. Livingston for many helpful suggestions throughout the progress of the work.

Some preliminary experiments dealt with the question whether there may be a definite correlation between moisture content of the soil and that of plant foliage at the time of the first appearance of wilting. The results showed clearly that no such correlation exists, at least under the midsummer conditions of southern Arizona. The rate of water loss from the aerial parts of the plant is frequently so high that loss of turgor occurs, even when the soil is approximately saturated. Such wilting has no relation either to the water-holding properties of the soil or to the condition of permanent wilting for the plant. It is without demonstrable effect upon the plant's capacity for absorbing water from the soil and the only injury resulting is that arising from the temporary interruption of growth. This phenomenon has therefore no direct relation to the main problem here attacked; its significance lies in the suggestion it offers as to the reason why, under the dry summer conditions of southern Arizona, certain plants practically cease their development as soon as a considerable extent of foliage has been formed.[24] These experiments were discontinued, but the data obtained in regard to the water content of foliage at the beginning of wilting suggested that further study of wilting might be profitable, and a series of determinations of foliar water content of fully turgid, just perceptibly wilted and permanently wilted plants was begun and continued throughout the summer. Both native plants grown in the open and cultivated species grown in pots were used. The detailed results would be out of place here and will appear in another connection. Some of the general results obtained with four species are summarized in Table I, which presents the maximum, minimum and average foliar water content found in openly exposed plants of the same age (six weeks), in the turgid, wilting and wilted condition.[25] For normal turgid plants, the higher percentages are from determinations made in early morning or upon cloudy, humid days; the lower figures were obtained by determinations made in mid-afternoon of clear days of low humidity and high temperature.

In these determinations, as in all others for which data are given in this paper, the water content was determined by collecting all normal functioning leaves exceeding 2.5 cm. in length into a tared screw-cap bottle, weighing immediately, drying for three days at 100-104° C, and reweighing without removing from the bottle. The errors of judgment necessarily involved in the selection of a given number or size of leaves to serve as a sample for moisture determinations are avoided when all the leaves of the plant are employed, and determinations made upon the entire foliage of the plant more correctly indicate the real condition with respect to foliar water, and therefore have a higher comparative value, than have those made by any other method, especially when turgid, wilting and wilted plants are to be compared. The work of Meschajeff[26] and others, especially that of Pringsheim,[27] has shown that there is a displacement of water from older to younger parts of the plant during wilting, and that the result of this redistribution is that the ratio of water to dry substance decreases in the various parts of the plant, in approximately equal ratio, until actual death of the older parts begins to occur. Determinations of the water content of the entire foliage avoid experimental error and misleading results due to this source, and hence should afford comparable results.

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[24] Livingston, B.E., Evaporation and plant development. Plant World **10**: 269-276. 1907. See especially page 275.

——— Evaporation as a climatic factor influencing vegetation. Proceed. International Conference on Plant Hardiness and Acclimatization. New York, 1907. Horticult. Soc. New York. Memoirs **2**: 43-54. 1910. Pages 48-49.

[25] Livingston and Brown [12] have recorded the variations in foliar water content in turgid plants for a number of species, accompanied by records of evaporation, in tables I to IV of their paper.

[26] Meschajeff, V., Ueber die Anpassung zum Aufrechthalten der Pflanzen und die Wasserversorgung bei der Transpiration. Bull. de la Soc. Imper. des Naturalistes de Moscou. No. 4, 1883. Summarized together with the earlier literature in Burgerstein [04], pages 228-230.

[27] Pringsheim, Ernst G., Wasserbewegung und Turgorregulation in welkenden Pflanzen. Jahrb. wiss. Bot. **43**: 89-145. 1906.

TABLE I

Water content of functioning leaves of openly exposed *Physalis*, *Zea*, *Martynia* and *Phaseolus* (six weeks of age), when fully turgid, just perceptibly wilted and permanently wilted, the foliage of each plant treated separately.

Name of plant and number of individuals employed.	Average water content, *per cent.*					
	On basis of dry weight			On basis of fresh weight.		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Physalis angulata var. *linkiana* Gray						
113 turgid plants	751	514	**598**	88.2	88.7	**86.0**
91 wilting plants	549	415	**503**	83.9	80.6	**84.3**
195 permanently wilted plants	461	302	**352**	81.8	75.0	**77.7**
Zea mays L.						
42 turgid plants	940	706	**804**	90.0	87.6	**88.8**
59 wilting plants	715	534	**675**	87.7	84.2	**87.1**
225 permanently wilted plants	590	425	**472**	85.5	81.0	**82.5**
Martynia louisiana Mill.						
29 turgid plants	672	481	**529**	87.0	82.4	**84.1**
18 wilting plants	490	357	**437**	83.0	78.1	**81.2**
41 permanently wilted plants	459	308	**347**	81.4	75.5	**77.6**
Phaseolus vulgaris L.						
18 turgid plants	735	546	**693**	88.0	84.5	**87.3**
16 wilting plants	612	483	**569**	85.0	82.8	**85.0**
30 permanently wilted plants	529	401	**486**	84.1	80.0	**82.9**

Several features are brought out by table I. Under the conditions prevailing throughout the experiments, which extended from July to October, the water content of turgid plants of the forms tested, growing in the open at Tucson, never rose very high above the point at which temporary wilting occurs. A decrease in water content amounting to 16.6 per cent. for *Physalis*, 15.6 per cent. for *Zea*, 17.5 per cent. for *Martynia*, and 18 per cent. for *Phaseolus* (all expressed as percentage of the average water content of turgid plants, calculated on basis of dry weight), appears to bring about temporary wilting of the younger leaves, in which parts form is maintained by turgor rather than by thickened cell walls or mechanical tissue. When grown in the open, wilting at midday and recovery in the late evening occur daily in these forms, to an extent scarcely equalled during the most prolonged drought in the more humid east. The loss of water necessary to produce permanent wilting, calculated as above, is 42 per cent. for *Physalis,* 40 per cent. for *Zea,* 34 per cent. for *Martynia,* and 30 per cent. for *Phaseolus,* as shown by the table. While the extremes show great overlapping, it must be borne in mind that varying proportions of fully grown to immature leaves must be very largely responsible for the great range of the figures given; a very great majority of the determinations made conformed very closely to the averages. The results give some support to the hypothesis previously advanced, that we have in the water content of the foliage a distinct indication of the condition of the plant as a whole, as has been surmised by Livingston and Brown ([12], pages 329-330), and further evidence in this direction will be adduced in the discussion of the wilting experiments to be described below.

It appears further from Table I that the four species here considered differ sensibly among themselves in the amounts of their foliar moisture content for corresponding physiological states, but these forms are too nearly alike in this respect to serve as a basis for any study of this point. Since different species frequently differ very widely in respect to the amount of water present in turgid leaves (Hedgcock [02], and others), it must follow that they differ correspondingly as regards their foliar moisture content at corresponding stages of wilting. Into this matter the present study does not enter.

The soils used were obtained on the Desert Laboratory domain and considered of a very pure sand, an "adobe" clay loam containing less than 0.1 per cent. of humus, and nineteen artificial mixtures of these two soils varying by increments of 5 per cent. of loam from 95 per cent. sand with 5 per cent. loam to 95 per cent. loam with 5 per cent. sand. The air-dry sand and loam were screened through a sieve of 2 mm. mesh and the various mixtures were then made up by volume in quantities sufficient for the work, being thoroughly mixed and again screened before using. Determinations of the moisture capacities and hygroscopic coefficients of the sand, the loam and the various mixtures, were made at the Desert Laboratory and repeated after my return to the University of Chicago, the method of Hilgard[28] being used in determining the moisture capacity upon a soil column one centimeter in height. These constants are shown in Table II. For comparison and reference, the calculated maximum available moisture for each soil—equal to the moisture-holding capacity minus the calculated "wilting coefficient" (Briggs and Shantz [12, *3,4*])—has been inserted in the table, as has also the hygroscopic coefficient obtained by calculation from the formula of Briggs and Shantz [12, *2,3*] (moisture holding capacity minus 21, multiplied by 0.234), the latter for comparison with the same coefficient as obtained by actual tests. The moisture equivalent, calculated from the moisture capacity by application of the formula of Briggs and McLane[29] has also been added.

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[28] Hilgard, E. W., Soils, their formation, properties and composition. New York. 1911. See page 209.

TABLE II

Moisture holding capacity, moisture equivalent, maximum available moisture, hygroscopic coefficient both observed and calculated, and "wilting coefficient," in per cent. of dry weight, for each of the 21 different soils used in the experiments

Soil components, *volume per cent.*		Moisture holding capacity, averages of 3 determinations	Calculated moisture equivalent	Hygroscopic coefficient		Calculated maximum available moisture	Calculated "wilting coefficient"
Sand	Loam			Observed	Calculated		
100	...	30.86	6.26	2.41	2.30	27.46	3.40
95	5	31.48	6.65	2.49	2.45	27.87	3.61
90	10	31.00	6.98	2.64	2.57	28.20	3.79
85	15	32.43	7.26	2.70	2.67	28.49	3.94
80	20	32.96	7.59	2.89	2.70	28.84	4.12
75	25	33.39	7.87	2.96	2.90	29.12	4.27
70	30	33.87	8.17	3.12	3.10	29.44	4.43
65	35	34.88	8.81	3.38	3.24	30.10	4.78
60	40	35.64	9.29	3.51	3.42	30.59	5.05
55	45	37.50	10.47	3.92	3.86	31.81	5.09
50	50	38.68	11.22	4.39	4.37	33.24	6.24
45	55	41.62	13.09	4.86	4.82	34.51	7.11
40	60	42.93	13.92	5.27	5.13	35.37	7.56
35	65	43.86	14.51	5.41	5.34	35.98	7.88
30	70	44.91	15.18	5.67	5.59	36.67	8.24
25	75	45.83	15.76	5.89	5.81	37.27	8.56
20	80	46.98	16.59	6.18	6.07	38.03	8.95
15	85	48.65	17.55	6.55	6.47	39.12	9.53
10	90	50.12	18.29	6.90	6.81	40.08	10.04
5	95	50.96	19.02	7.13	7.01	40.63	10.33
...	100	52.64	20.09	7.51	7.40	41.73	10.91

The containers used in these tests were cylinders of tinned sheet-iron, 11.5 cm. deep and 7.5 cm. in diameter, having small perforations near the base and through the bottom to secure ventilation and drainage while the plants were growing. Each one contained 550 g. of air-dry soil. In the experiments with *Xanthium*, *Physalis* and *Martynia*, young seedlings were transferred from the open ground to the cylinders as soon as they could be recognized with certainty, usually when not more than two foliage leaves had developed. The other forms used were grown from seeds planted in the cylinders. The soil was left uncovered until from 24 to 36 hours before the plants were to be used, when they were watered and dripping was allowed to cease. The upper surface of the soil and the perforations in the cylinder were then carefully covered with plastiline, a composite modeling clay made up with a nonvolatile and nonhardening oil. Plants grown in this manner show no abnormalities in the distribution of the root system, do not appear to suffer from lack of oxygen during the short period in which the cylinder is sealed, and the stems are not injured by contact with the seal if the clay used is suitable.[30]

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[29] Briggs, Lyman J. and McLane, John W., The moisture equivalents of soils. U.S. Dept. Agric. Bur. Soils. Bull. 45. 1907.

Soil samples were taken from top to bottom of the cylinders, 2 cm. from the center, using an 18 mm. cork borer and discarding the upper and the lower 2.5 cm. of soil column thus obtained. The accuracy of this method has been repeatedly tested by check weighings of the entire remaining soil content of sampled cylinders and has been found entirely adequate for the present purpose.

As has been stated (page 12), leaf moisture determinations were in all cases made from all the living normal leaves of the plant having a length greater than 2.5 cm., those having dead or dying tips, wounds made by insects, or other abnormalities being discarded. The use of entire plants or of the entire aerial parts, in moisture determinations, would have introduced a large and highly variable error under the conditions that prevailed during the experiments. The death of the older leaves proceeds at varying rates and a group of plants of the same age shows very large differences when the extent of the foliage is compared with that of the woody parts. Since the water of stems forms only about one-half, while that of leaves constitutes from four-fifths to nine-tenths of the fresh weight, comparisons based on weights of the entire aerial portion of the plant can have no meaning. On the other hand, no such variable source of error is introduced when the water content of functioning leaves of the same age and degree of development is used for the comparisons.

Leaf and soil samples were in all cases collected into tared screw-cap bottles. These were immediately tightly closed, weighed, dried at 98-102° C. (for three days longer, till a constant weight was attained), and again weighed without removal from the bottles.

Somewhat detailed descriptions of a number of wilting experiments will now be presented. These are selected from, and are fairly representative of, a considerably larger number.

Series I. The purpose of this experiment was to compare the soil moisture content found at permanent wilting in the open, under measured conditions of evaporation, with this moisture content found for the same series of plants when subsequently wilted in a moist chamber. At the first wilting the plants stood upon a table in the open, fifteen meters from any

It appears that some of the plastiline upon the market contains a large amount of sulphur, which renders it unsuited to this sort of use.—The material is generally to be obtained from dealers in artists' supplies.

building, where they received full insolation throughout the day; at the second wilting they were placed in a moist chamber. The latter was a tight, glass-walled, glass-roofed, rectangular box, 1.5 x 1 x 0.75 m., so placed under a roof without walls that it was not reached by direct sunlight, while free circulation of air took place about it. The air temperature of this chamber had a daily range almost equalling that outside, while the percentage of relative humidity ranged only between 96 and 99.5.

In this series, as also in the others which are to follow, data descriptive of the aerial environment of the plants were carefully recorded at hourly intervals. Readings of temperature were obtained from shaded thermometer and recording thermograph, those of humidity from a hygrograph, and those of the evaporating power of the air from the porous cup atmometer. The atmometer readings have been corrected to the Livingston standard,[31] and are always given, throughout this paper as hourly rates (cubic centimeters) for the preceding hour. The results are shown in tables III and IV.

Fifty plants of maize were employed in this series. They were eight weeks old, growing in a mixture of half sand and half loam, this soil having a calculated wilting coefficient (see table II) of 6.24 per cent. At the first wilting, in the open (table III), soil samples were obtained from all the cultures, and leaf moisture determinations were made for sixteen plants. Leaf moisture and soil moisture determinations were made for all of the remaining thirty-four plants at the second wilting, in the moist chamber (table IV).

In this series the data of wilting in the open show a very marked departure of the observed from the calculated soil moisture residue, which leads to a high ratio of the observed to calculated. The corresponding results of the second wilting, in the moist chamber, show a very close agreement and the derived ratios do not here depart greatly from unity. In this respect the experiment fairly represents a number of similar series, in which altogether twenty-one soils, having calculated wilting coefficients ranging from 4.12 to 10.04 per cent. of their dry weight, were tested in this way. While the details of the results vary with the aerial conditions prevailing throughout the period of the experiment, each of these series shows observed soil moisture contents at permanent wilting markedly higher than the corresponding calculated figures, for every wilting occurring in the open; but essential agreement of observed with calculated soil moisture content for subsequent wiltings occurring in the moist chamber.

Series II. The purpose of this series was to compare the calculated wilting coefficient, for the same soil as that used in series I, with the actual

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[31] Livingston, B.E., A rotating table for standardizing porous cup atmometers. Plant World 15: 157-162. 1912. Other references describing various methods of standardization are there given.

TABLE III (series I)

Leaf moisture content and ratio of observed to calculated wilting coefficient at permanent wilting for *Zea*, the plants eight weeks old at beginning of experiment.

First wilting, in open, Oct. 2.

Hour	Temperature	Relative humidity	Evaporation rate for preceding hour	No. of plants wilting	Ratio of actual to calculated soil moisture residue at wilting	Leaf moisture,[32] *percentage* of Dry weight	Leaf moisture,[32] *percentage* of Fresh weight
	degrees C.	*per cent.*	*cc.*				
6	24.0	39	1.6	..	....	...	....
7	26.0	30	2.1	.	....	...	....
8	27.0	31	2.5	2	1.19	...	....
9	28.6	27	3.9	9	1.36	482	82.8
10	29.5	22	3.1	7	1.52	492	83.1
11	30.3	22	4.3	8	1.42	490	83.1
12	30.1	21	6.0	6	1.58	485	82.9
13	30.1	23	4.1	4	1.58	481	82.8
14	30.4	21	3.5	6	1.49	459	82.1
15	28.6	25	3.4	6	1.42	501	83.4
16	27.9	29	2.3	2	1.36	503	83.4
Average.						484	82.9

TABLE IV (series I, continued)

Second wilting, in moist chamber, Oct. 8

Hour	Temperature	Relative humidity	Evaporation rates, for preceding hour	No. of plants wilting	Ratio of actual to calculated soil moisture residue at wilting	Leaf moisture[32] *percentage* of Dry weight	Leaf moisture[32] *percentage* of Fresh weight
	degrees C.	*per cent.*	*cc.*				
6:30	24.2	99	0.1	..	....	...	....
7:30	25.1	99	0.1	..	....	...	....
8:30	26.7	98	0.2	8	1.02	504	83.4
9:30	28.1	97	0.2	6	1.05	455	82.0
10:30	28.8	97	0.2	7	1.10	516	83.8
11:30	29.7	97	0.2	..	....	...	....
12:30	30.1	96	0.3	5	1.14	476	82.7
13:30	31.6	96	0.2	5	1.02	503	83.4
14:30	31.5	96	0.3	3	1.03	490	83.1
Average.						491	83.1

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[32] During the first part of the experiment (table III) two wilted plants were used hourly for leaf samples and the others were revived. During the second part (table IV) all plants which had wilted during the preceding hour were used for leaf samples. The average percentages of leaf moisture given are thus derived from two plants in each case, in the first table, and from the corresponding number of wilting plants stated for the hour in question, in the second.

coefficients found when the same plants were repeatedly wilted under various conditions. In order that the plants might undergo as many wiltings as possible, soil samples,—the taking of which of course injures the root system of the culture,—were here mainly dispensed with. Instead of the method of sampling, a method of calculation was employed. The original weights of cylinder and seal and of the dry soil were recorded for each of the thirty-one cultures, and from these constants and the total weights obtained at each wilting, the weights of soil water and plant were readily derived in each case. To arrive at a satisfactory approximation of the soil moisture content, four representative cultures were discontinued at each wilting and were used as samples for estimating the weights of the plants themselves in the remaining cylinders. From these four sample cultures the plants were removed by carefully screening the soil and were then weighed. The plant weights thus obtained varied less than a single gram from their average in every case. This average plant weight was assumed to represent the weight of each plant in the undisturbed cultures, and the deduction of this weight from each of the combined weights of soil water and plant already derived gave the approximate weights of soil water in each case. This method no doubt produces a slight error due to unequal development of the plants in the different cultures, but with a total weight of 600 grams of soil this error in the percentage of soil moisture is surely no greater than is inherent in the method of sampling by means of the cork borer, which was used throughout as a control.

The plants (31 in all) were 39 days old at the first wilting and were wilted seven times. Wiltings 1, 2, 3, 4, and 6 occurred in the open as described for series I, wilting 5 occurred in a lath shelter (described by Brown [12], page 123), which allowed one-half the sunlight to pass, while wilting 7 occurred in the moist chamber already described. The plants stood in the open from the time of planting until three days before the first wilting; they were then sparingly watered, after which the tops of the cylinders and all openings were sealed with plastiline. As soon as permanent wilting appeared to be established in any plant, the latter was transferred, after weighing, to the moist chamber. Failure to regain turgor after twenty-four hours in this chamber was taken to denote that permanent wilting had indeed been attained. The seal was then opened, water added to the soil, and the plant returned to the chamber to recover turgor. As soon as this occurred it was again brought into the open.

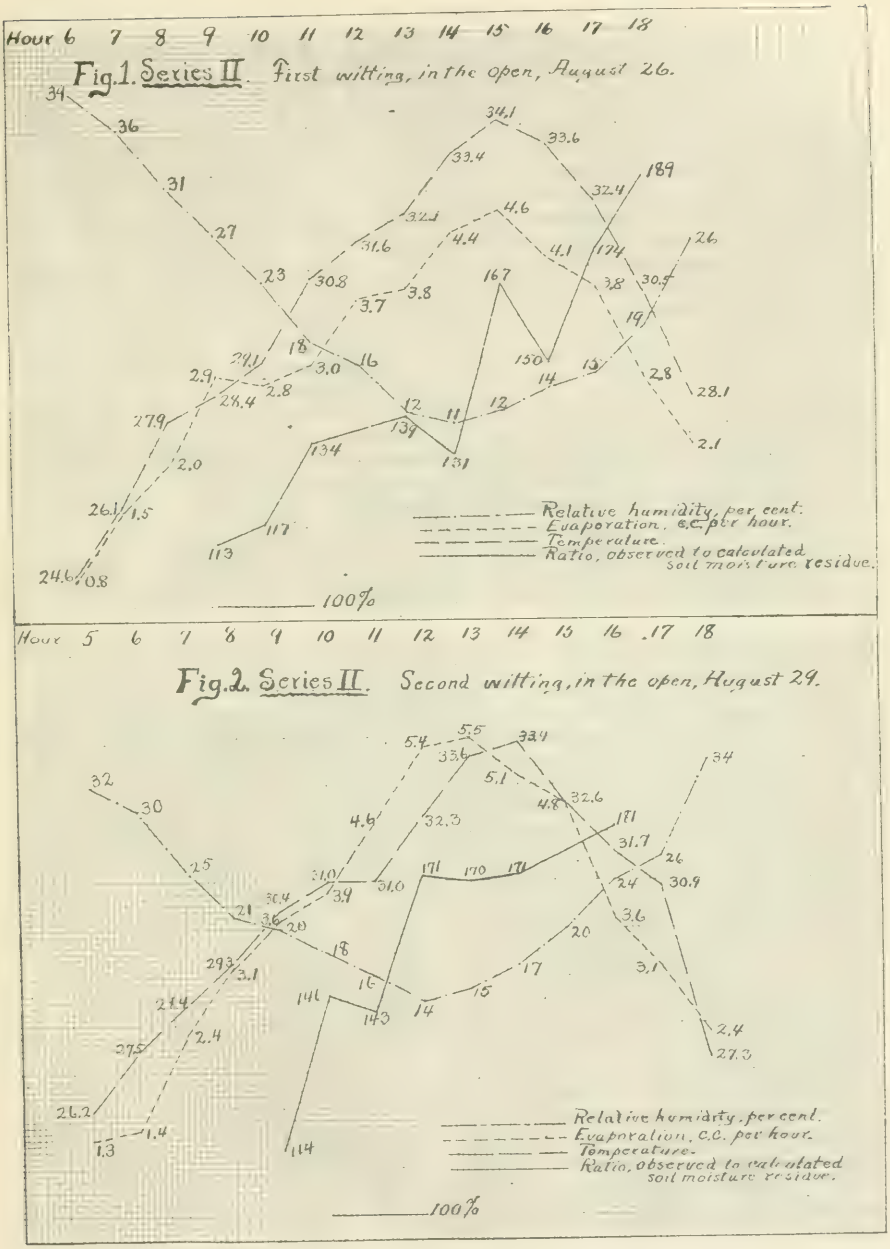
Figures 1 to 7 show the results of the seven wiltings in the form of graphs, in which are recorded, for each hour of the days of wilting, the instrument readings, the average soil moistures and the ratio of average soil moisture to calculated "wilting coefficient" for the plants attaining permanent wilting at each hour. Table V shows the water content of leaves, in percentage of dry weight, for each of the plants used for leaf samples at any wilting.

TABLE V (series II)

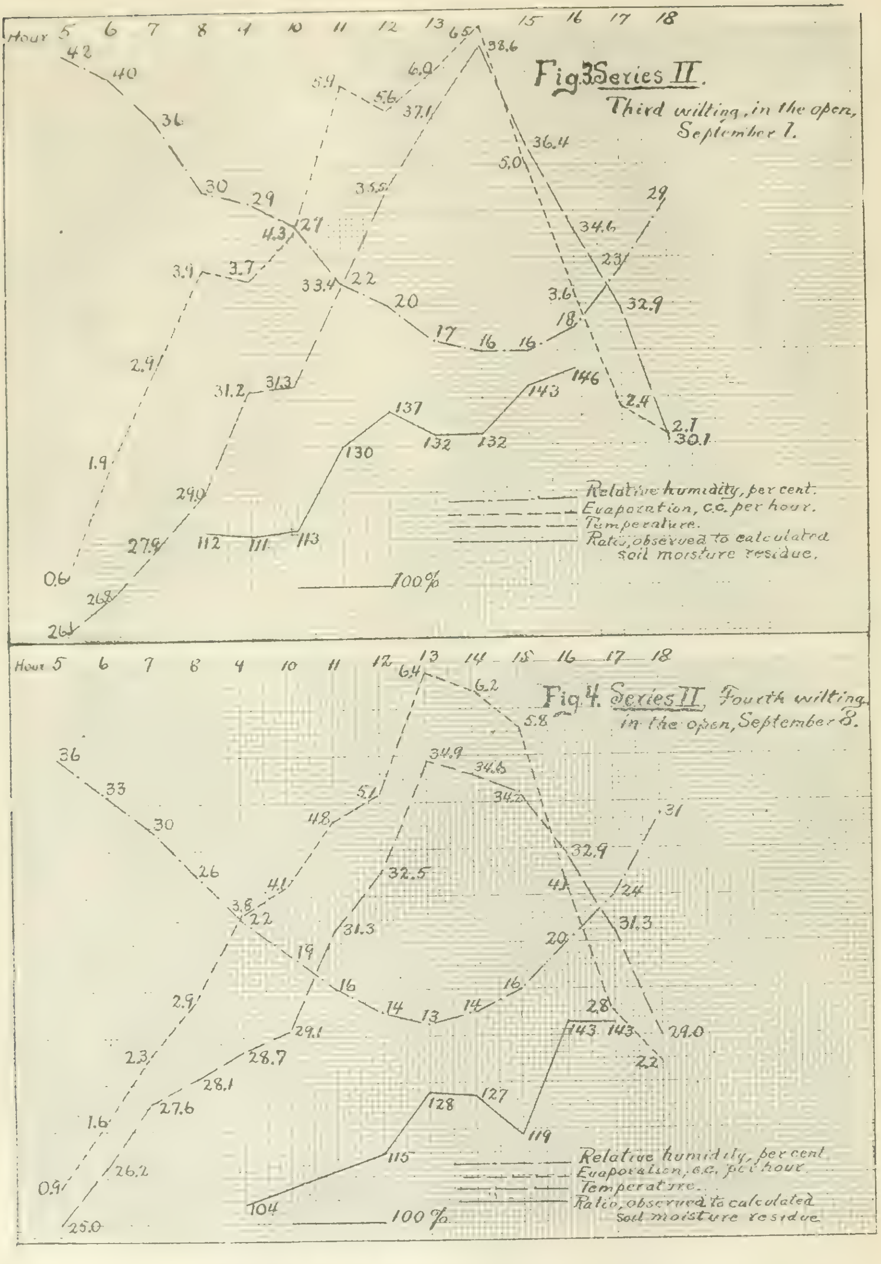
Foliar water content of *Zea*, from seven successive wiltings, the plants 39 days old at first wilting.

Wilting No.	Wilted in	Sample No. 1 Per cent. of Dry wt.	1 Per cent. of Fresh wt.	2 Per cent. of Dry wt.	2 Per cent. of Fresh wt.	3 Per cent. of Dry wt.	3 Per cent. of Fresh wt.	4 Per cent. of Dry wt.	4 Per cent. of Fresh wt.	Average Per cent. of Dry wt.	Average Per cent. of Fresh wt.
1	Open....	545	84.5	560	84.9	513	83.7	526	84.0	536	84.4
2	Open....	462	82.2	533	84.2	493	83.2	470	82.5	489	83.0
3	Open....	450	82.1	426	81.0	457	82.1	439	81.5	445	81.6
4	Open....	459	82.2	444	81.6	476	82.7	443	81.6	455	82.0
5	Open....	533	84.2	472	82.5	463	82.3	454	82.0	480	82.7
6	Shelter...	445	81.7	507	83.6	477	82.7	...	....	476	82.6
7	Open....	532	84.2	456	82.1	589	85.5	516	83.8	523	84.0

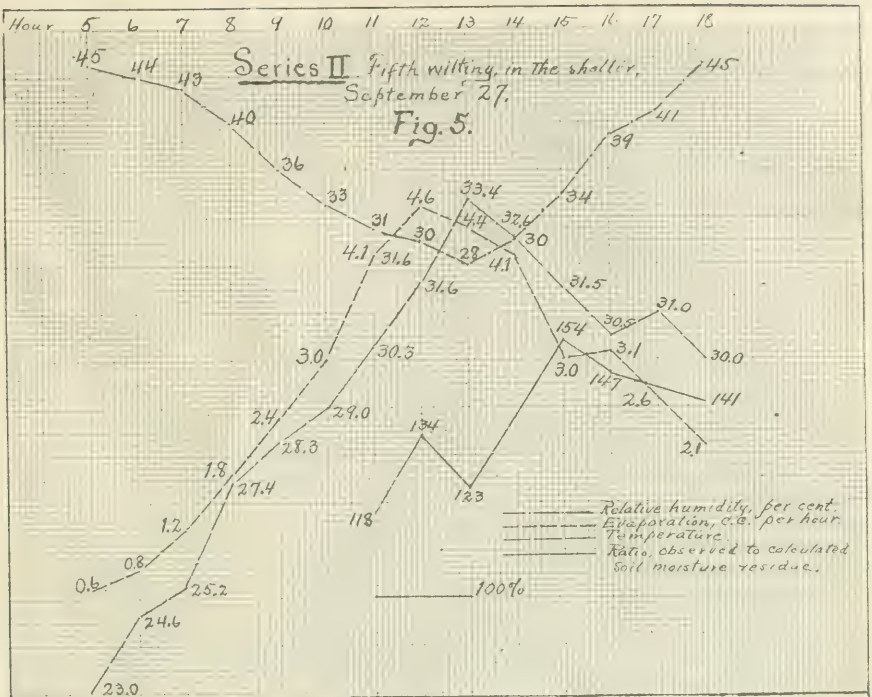
Several facts brought out by the results of this series may be briefly stated here. The soil moisture determinations for the first four wiltings, (figs. 1-4), practically without exception, yield results very materially higher than the calculated numbers obtained for these soils. When this became apparent in the preliminary experiments, it was thought to be attributable to the heating of the soil by direct sunlight, but when methods which effectually prevented such heating were employed, or when the plants were wilted in the lath shelter, in which no heating of the soil by the sun occurred (although the air temperatures equalled those outside), it was found that the observed soil moisture contents showed practically no decrease. The departure of observed from calculated residues is in the majority of cases very considerable, ratios of observed to calculated residues ranging from 1.02 to 1.91 in the four wiltings here under consideration. For each of these days of wilting, it is further noticeable that the observed soil moisture residue is always nearest the calculated figure for plants attaining the permanently wilted condition in the early morning hours. With the increase in evaporation rate which accompanies rise of temperature and decrease in humidity, the curve representing the ratio of observed to calculated soil moisture residue rises in fairly regular fashion until the maximum evaporation rate for the day has been attained. The ratio then remains practically stationary for the remainder of the day, in two cases falling off slightly to rise again later in the afternoon (figs. 1 and 4), but in each of the four wiltings those plants which became wilted

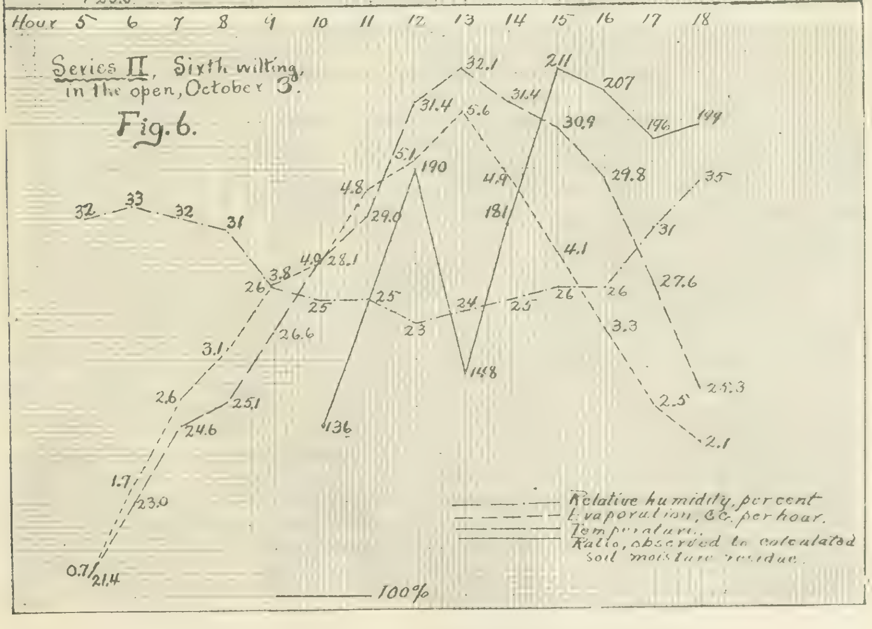


Graphs of series II, first and second wiltings.

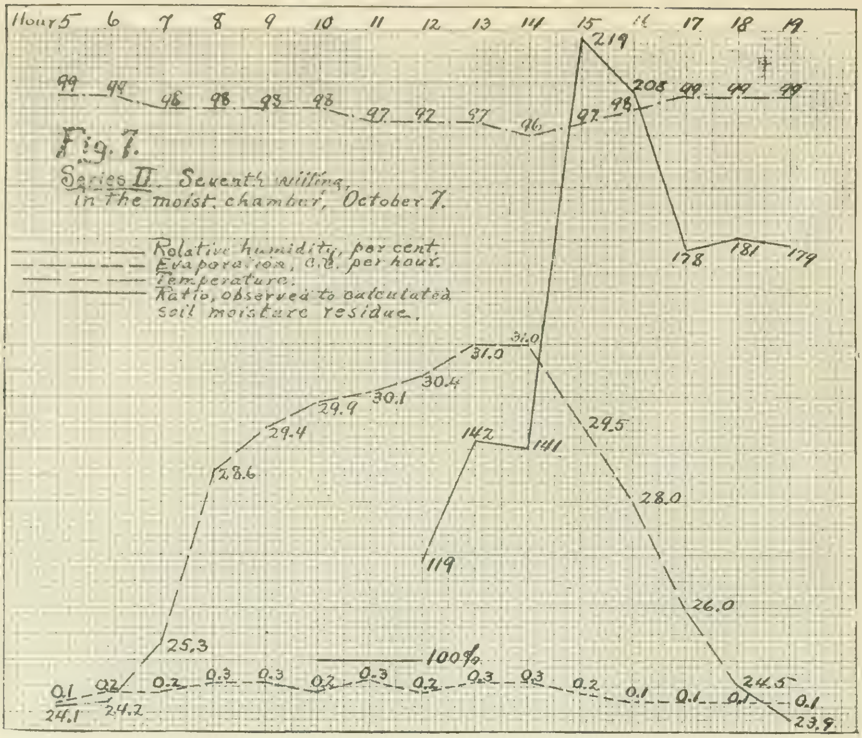


Graphs of series II, third and fourth wiltings.





Graphs of series II, fifth and sixth wiltings.



Graphs of series II, seventh wilting.

at the last hour of wilting show an average ratio higher than that for any group which precedes them. The curve for the fifth wilting, which occurred under the less rigorous conditions of the partial shelter, shows some irregularities but is recognizably of quite the same general type as its predecessors.

It is also evident from these results that repeated wiltings in the open are without discoverable effect upon the amounts of the soil moisture residue found at subsequent wiltings. The range of extremes in the ratio of observed to calculated soil moisture residues is almost identical, namely from 1.13 to 1.90, in the case of the first two wiltings. In wiltings 3, 4, and 5 there is a like identity in so far as the lower limit is concerned, while the extremes reached by plants wilting in the later hours of the day are materially lower, nowhere exceeding 1.52. When one notes the essential similarity of the environmental conditions prevailing on the days of wilting, it appears that the similarity of the curves representing these ratios find therein an explanation.

For the sixth and the seventh wiltings, the curves representing the ratios of observed to calculated soil moistures at wilting have a very different character. The departures of observed from calculated soil moisture residue are of an entirely different order of magnitude; the greater number of wiltings occur with soil moistures which are 150 to 200 per cent. of the calculated values; and there is no discoverable relation between the results found for any given hour of the day and the environmental conditions prevailing at that time. Discussion of the causes leading to this result will be postponed until the data from series III, which was planned to throw light upon this point, have been presented.

The results of determinations of foliar water content show that in *Zea* the water content of the leaves at permanent wilting ranges, for the 31 plants of this experiment, between 85.5 and 81.0 per cent. of the dry weight, while the range of averages for the groups is somewhat less, or from 84.4 to 81.6 per cent. This accords closely with the general average of 82.5 per cent. shown by 225 plants of this species (table I), and makes it evident that the permanently wilted condition is one characterized by a rather definite foliar water content. It is further apparent that, within the time limits of this experiment, the increase in age of the plants—which were 39 days old at the first wilting and 80 days at the seventh—is without effect upon the amount of this content, since the averages for the first and the last wiltings are 84.4 and 84.0 per cent. respectively.

Series III. This series was planned to serve as a check upon the results of series II, using a soil of somewhat lower water capacity, and to show the influence of repeated wiltings in the moist chamber upon the percentages of water in soil and leaves at the time of wilting.

The series employed forty plants of maize grown in a mixture of 60 per cent. sand and 40 per cent. loam, having a calculated "wilting coefficient" (formula of Briggs and Shantz [12, *3,4*]) of 5.05. The plants were wilted seven times, the first three wiltings occurring in the open, the fourth in the lath shelter, and the last three in the moist chamber. The plants were six weeks old on the day of the first wilting and the procedure was precisely as in series II. The detailed results are shown by the graphs of figures 8 to 14, while the foliar water contents for four samples taken at each wilting are shown in table VI.

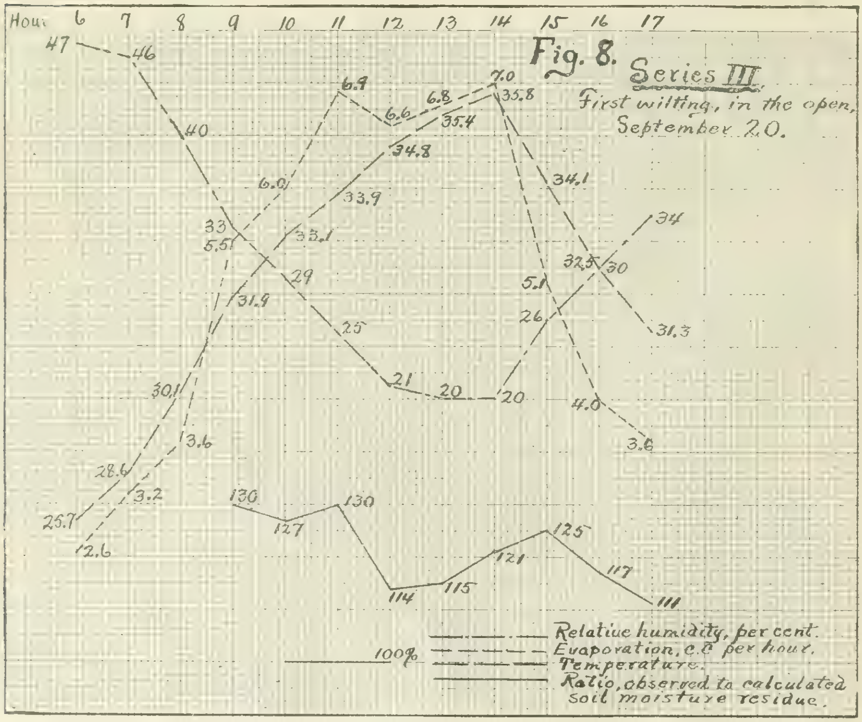
The series of wiltings from which the results shown in figures 8-14 were derived present some marked differences when compared with the corresponding graphs for wiltings in the open in series II. While the soil moisture residues for the wiltings in the open everywhere considerably exceed the calculated figures, they are nowhere so high as is the case in that series. In only one of the four wiltings in the open (fig. 11) does the curve representing the ratio of observed to calculated soil moisture residue rise steadily with the rise in rate of evaporation; in the earlier

TABLE VI (series III)

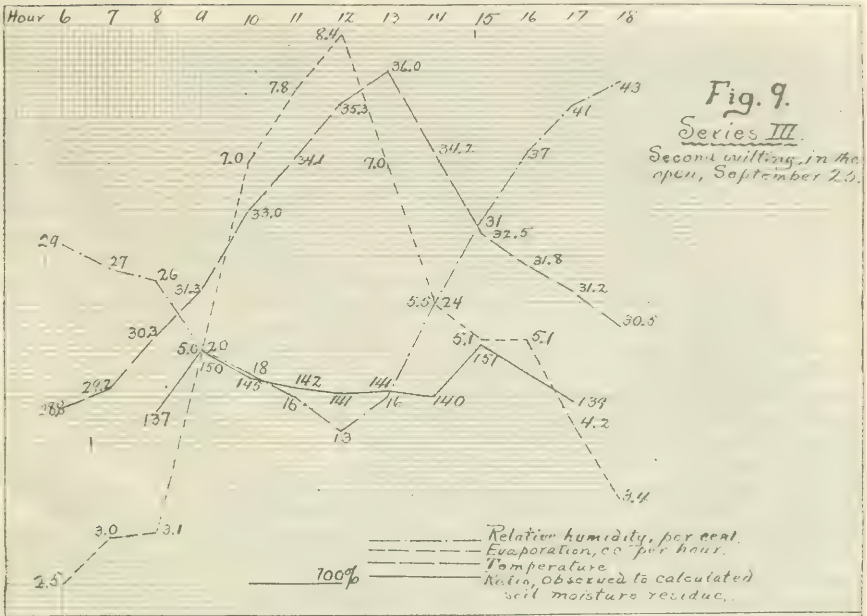
Foliar water content of *Zea*, from seven successive wiltings, the plants six weeks old at first wilting.

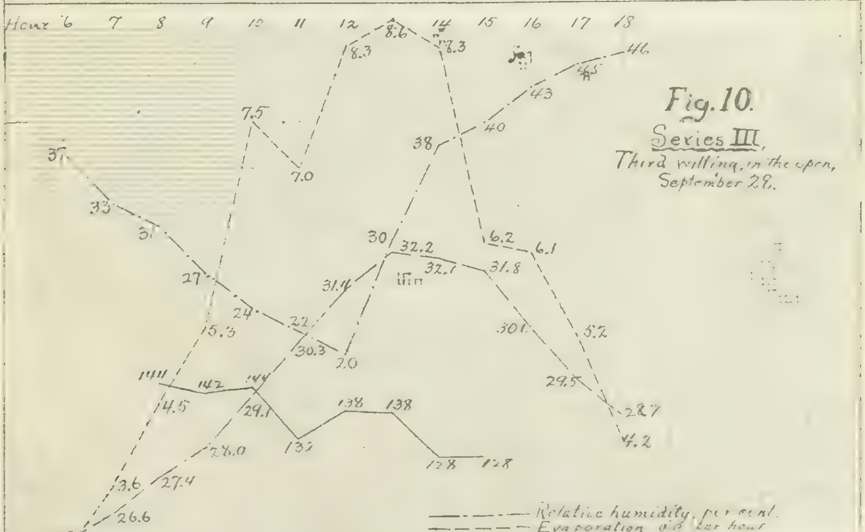
Wilt No.	Wilting in	Sample No. 1 Per cent. of		2 Per cent. of		3 Per cent. of		4 Per cent. of		Average Per cent. of	
		Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.
1	Open....	518	83.8	562	84.9	499	83.3	513	83.7	523	84.0
2	Open....	559	84.9	523	84.0	506	83.5	556	84.8	536	84.3
3	Open....	559	84.9	500	83.6	503	83.4	506	83.5	519	83.9
4	Shelter...	496	83.2	505	83.6	533	84.2	502	83.4	510	83.6
5	Chamber.	489	83.0	501	83.4	506	83.5	507	83.6	501	83.4
6	Chamber.	526	84.0	491	83.1	513	83.7	541	84.4	518	83.6
7	Chamber[33]	...	....	...	....	...	....	...	....	513	83.7

[33] Only the average of thirteen plants taken together.

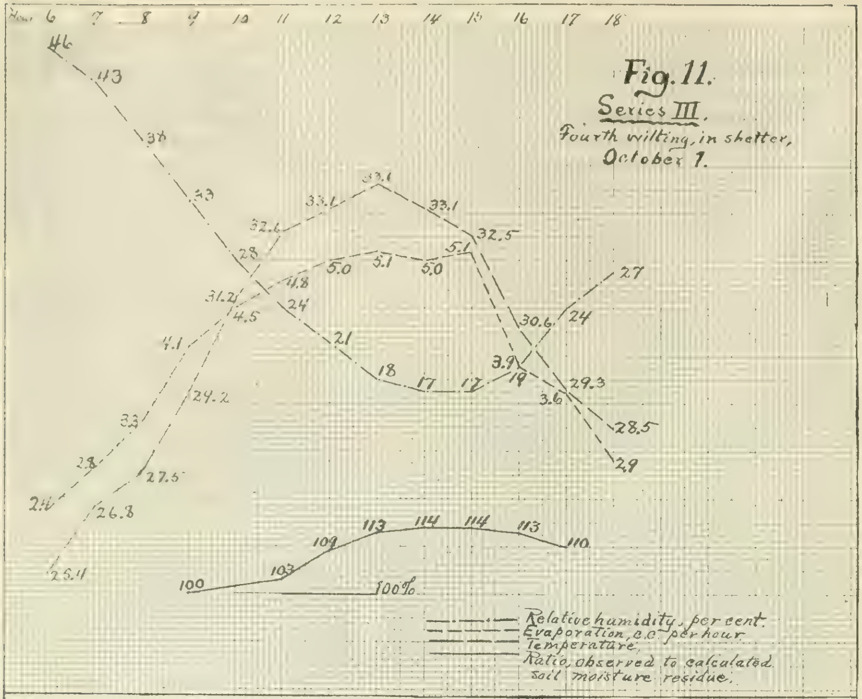


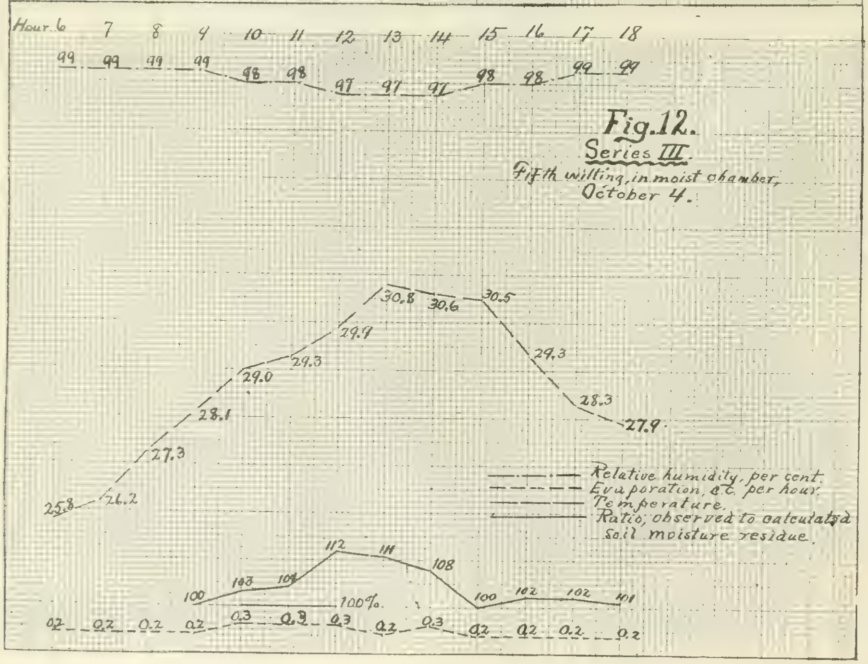
Graphs of series III, first wilting.





Graphs of series III, second and third wiltings





Graphs of series III, fourth and fifth wiltings.

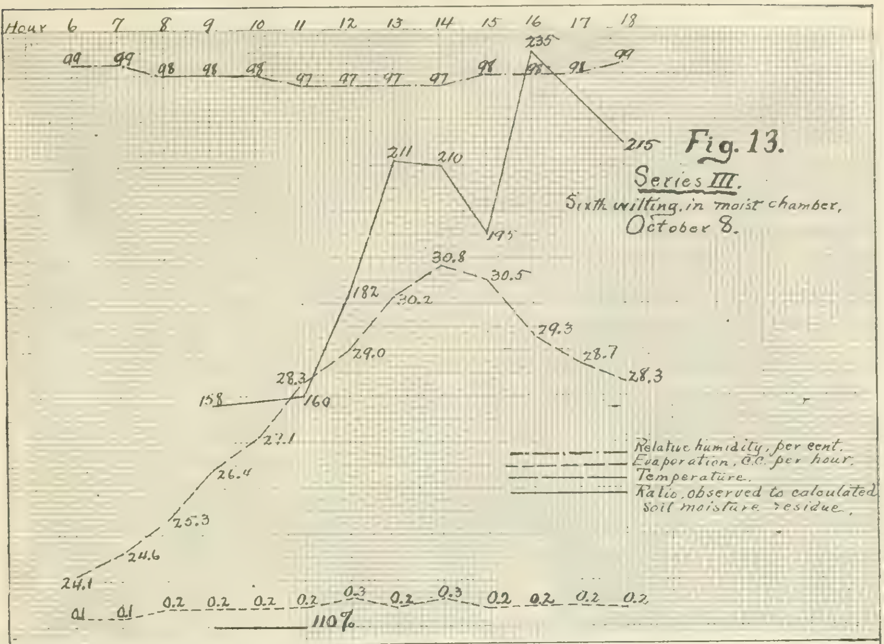


Fig. 13.

Series III.
Sixth wilting, in moist chamber,
October 8.

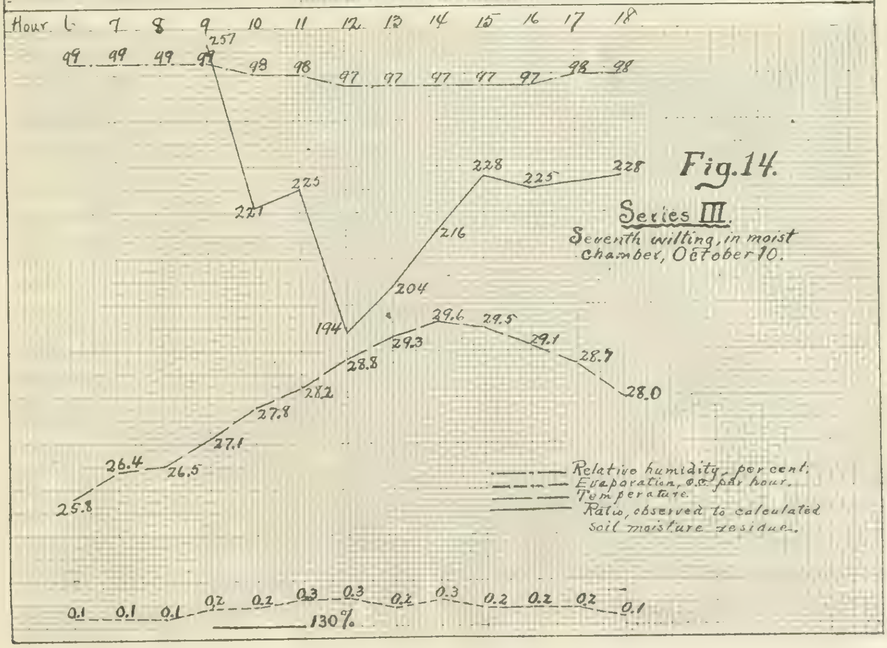


Fig. 14.

Series III.
Seventh wilting, in moist chamber, October 10.

Graphs of series III, sixth and seventh wiltings.

wiltings the soil moistures found for plants at the first hour of wilting equalled or exceeded those found later (figs. 8, 9, and 10). These three wiltings also agree fairly well among themselves as regards the mean soil moisture residue, which for all three lies between 1.25 and 1.40 times the calculated "wilting coefficient" of 5.05 per cent. Under the moderate conditions of evaporation prevailing at the fourth wilting, in the lath shelter, this mean sinks to 1.08, the highest ratio found for the day being 1.13 (fig. 11). At the fifth wilting, in the moist chamber, the observed soil moisture residues still more closely approach the calculated figures, while a comparison of the results of wiltings 4 and 5 shows conclusively that no injury to the absorbing and transporting system of the plants had resulted from the repeated wiltings in the open.

When the results of the fifth, sixth, and seventh wiltings, which occurred in the moist chamber and under closely similar conditions (figs. 12, 13, and 14), are compared, it will be seen that while the ratio of observed to calculated soil moisture residue very closely approaches unity for the first of these wiltings, it is nowhere less than 1.58 in the next and everywhere in excess of 2.00 in the last. It was evident that a very material decrease in the capacity of the plants to utilize the soil water had occurred as a result of the first wilting in the moist chamber. Microscopic examination of the roots of such plants showed that a condition of complete plasmolysis is attained in the very great majority of roothairs of *Zea* at the time at which permanent wilting occurs in the moist chamber, and further observations of such roothairs made it clear that functional activity is never restored in more than a very small percentage of such hairs. The very high soil moisture residues found at second and third wiltings are therefore due to the very great reduction in area, and therefore in efficiency, of the absorbing system, consequent upon general destruction of roothairs at the first wilting.

It will be noted also (from table VI) that the leaf samples from these seven wiltings show a moisture content ranging from 83.4 to 84,3 per cent. of the dry weight, from which it is apparent that the magnitude of this content is unaffected by the conditions under which wilting occurs.

Preliminary experiments in which plants were allowed to wilt under otherwise identical conditions, but in soils of different water-holding capacities, seemed to indicate that while wilting in the open always exhibited soil moisture residues somewhat higher than the calculated figures, this lack of agreement was most marked in the case of sand and became less pronounced as the percentage of loam in the mixtures increased. Out of a larger number of series prepared for the examination of this point, the series which will here be termed IV, V, and VI have been selected for description, being fairly representative of the results obtained.

Series IV. The purpose of this experiment was to determine the relations of the theoretical to the observed soil moisture residues at permanent wilting, for several soils, when the plants were twice allowed to wilt, once in the open and once in the moist chamber. Eleven soil mixtures were made up, beginning with pure sand and passing by 10 per cent. additions of loam to pure loam. For the first experiment, five cylinders of each of the eleven mixtures, each containing 700 g. of soil, were planted with maize and the plants were allowed to grow in the open until six weeks old, when they were thoroughly watered and sealed with plastiline. The first wilting occurred three days later, on October 1. Of the group of five plants in each grade of soil, three were used for leaf samples while soil samples were taken from all. There thus remained two plants of each group for use in the second wilting, which occurred in the moist chamber, on October 7. The detailed results are shown in tables VII and VIII, which state for each hour of the days of wilting the atmospheric conditions (these data being for the preceding hour), the kind of soil mixture in which wilting occurred, the number of plants wilted ,and the ratios of observed to calculated soil moisture residues.

It will be observed from tables VII and VIII that the range of magnitude of the ratios of observed to calculated soil moisture residues is very great in the case of every soil mixture, and that there is much overlapping, the latter being so great that no detailed statistical treatment of these ratios could be of any avail without a much larger body of data. It is clearly apparent, however, that there is a strong tendency of the higher ratios to occur with the more sandy soils and of the lower ratios to occur with mixtures containing less sand. To illustrate this, it may be observed that the four minimal ratios greater than 1.07 all occur with more than 50 per cent. of sand, while the six minima less than 1.07 are restricted to soil mixtures with less than 60 per cent. of sand. Similarly the four maximal ratios greater than 1.85 all occur with over 50 per cent. of sand, and the four maxima below 1.60 are found in mixtures with less than 60 per cent.

It may also be noted (table VIII) that the average moisture content of the wilting leaves was practically the same whether wilting in the open or in the moist chamber.

Series V. In this series plants of *Xanthium commune* Britton and *Martynia louisiana* Mill. were subjected to treatment and wilting in a manner similar to that used with maize in series IV, but in the case of *Xanthium* only six soil mixtures were employed; namely, pure sand, 80, 60, 40, and 20 per cent. sand, and pure loam. Forty-two plants of *Xanthium* were grown from seed in the open, in as many cylinders, seven in each of the six soils. Five week-old plants of *Martynia* were transplanted into as many cylinders of each of the eleven soil mixtures used in series IV. The

TABLE VII (series IV)

Ratios of observed to calculated wilting coefficients for *Zea* in graded sand-loam mixtures, the plants six weeks old at first wilting.

First Wilting, in the open, Oct. 1.

Hour	Temperature,	Relative humidity	Evaporation rates, for preceding hour,	No. of plants wilting	Sand in soil mixture	Ratio of observed to calculated soil moisture content at permanent wilting
	degrees C.	*per cent.*	cc		*per cent.*	
7:30	26.8	43	1.8	..	..	....
8:30	27.5	38	2.0	..	..	....
9:30	29.2	33	2.6	..	..	....
10:30	31.2	28	3.7	2	40	1.23
				1	60	1.57
11:30	32.0	24	4.2	1	30	1.05
				1	40	1.29
				1	50	1.03
				1	70	1.07
12:30	33.1	21	5.8	1	40	1.39
				1	50	1.16
13:30	33.6	18	6.1	3	00[34]	1.36
				1	10	1.73
				1	30	1.17
				2	50	1.17
				1	60	1.47
				1	70	1.37
				1	80	1.38
				1	90	1.37
14:30	33.1	17	6.2	1	00[34]	1.27
				1	10	1.24
				3	20	1.33
				1	30	1.35
				1	50	1.57
				2	60	1.62
				1	80	1.49
				2	100	2.31
15:30	32.5	17	6.1	1	10	1.24
				2	20	1.18
				1	30	1.43
				1	40	1.82
				1	80	2.25
				1	90	1.65
				1	100	2.12
16:30	30.6	19	5.4	1	60	1.03
				1	70	1.42
				1	100	1.30

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[34] Loam without admixture of sand.

plants were kept in the open with daily watering until they were seven weeks old, when they were sealed in preparation for the first wilting, which occurred four days later, on September 25. The first and second wiltings occurred in the open, the third and fourth in the moist chamber.

The detailed results of this series demand too much space for presentation here; they are in all respects confirmatory of those obtained with maize in series IV. They bring out with special clearness the fact that the largest departures of the observed from the calculated soil moisture contents at wilting occur in the soils of lower water-holding capacity,

TABLE VIII (series IV, continued)

Second wilting, in moist chamber, Oct. 7 and 8

Hour	Temperature	Relative humidity	Evaporation rates, for preceding hour	No. of plants wilting	Sand in soil mixture	Ratio of observed to calculated soil moisture content at permanent wilting
	degrees C.	*per cent*	cc.		*per cent.*	
8:30	28.7	98	0.3	1	40	1.[illegible]
				2	50	1.15
				1	60	1.36
				1	90	1.48
9:30	29.4	98	0.3	.	. .	. . . .
10:30	30.0	98	0.2	1	10	1.00
				1	20	0.98
				1	30	1.47
				1	70	1.35
				1	80	1.45
11:30	30.3	97	0.2[35]	.	.	.
7:30	25.1	99	0.1	.	. .	. .
8:30	26.7	98	0.2	1	00[36]	1.05
				2	20	1.13
				2	40	1.27
				1	100	1.68
9:30	28.1	97	0.2	1	10	1.15
				2	70	1.87
10:30	28.8	97	0.2	.	.	.
11:30	29.7	96	0.3	1	40	1.49
12:30	30.1	96	0.3	2	20	1.30
				2	30	1.26
				1	70	1.34

Average foliar water content,	*per cent.* of dry weight	*per cent.* of fresh weight
First wilting (table VII)	491.4	83.1
Second wilting (table VIII)	506.0	83.5

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[35] No further wilting on Oct. 7.

[36] Loam without admixture of sand.

while the observed soil moisture residue steadily approaches the calculated figure with increase in this soil property. This point is brought out by table IX, while table X gives the water-content of the leaves at the different wiltings.

TABLE IX (series V)

Ratios of calculated to observed soil moisture contents at permanent wilting, for *Martynia* and *Xanthium* repeatedly wilting, under various conditions, the plants seven weeks old at beginning of experiment.

Soil mixture, *per cent* of sand	*Martynia*				*Xanthium*			
	Wilting in open		Wilting in moist chamber		Wilting in open		Wilting in moist chamber	
	First wilting	Second wilting	Third wilting	Fourth wilting	First wilting	Second wilting	Third wilting	Fourth wilting
100	1.73	1.42	1.39	1.80	1.94	1.83	1.61	2.30
90	1.62	1.44	1.39	1.77				
80	1.70	1.47	1.42	1.61	1.91	1.82	1.53	2.22
70	1.70	1.55	1.40	1.65				
60	1.56	1.53	1.51	1.68	1.69	1.63	1.30	1.84
50	1.29	1.33	1.22	1.52				
40	1.15	1.18	1.09	1.39	1.39	1.37	1.05	1.49
30	1.10	1.17	1.04	1.33				
20	1.16	1.13	1.06	1.25	1.38	1.26	1.03	1.42
10	1.15	1.10	1.06	1.35				
00	1.14	1.05	1.05	1.37	1.32	1.12	1.10	1.27

TABLE X (series V, continued)

Average foliar water content of *Martynia* and *Xanthium* repeatedly wilted, under various conditions.

Plant	No. of wilting	No. of plants used	Foliar moisture content	
			On basis of dry weight, *per cent.*	On basis of fresh weight, *per cent.*
Martynia	1	11	391.8	79.6
	2	11	388.2	79.3
	3	11	380.0	79.1
	4	22	396.1	79.8
Xanthium	1	6	345.1	77.5
	2	6	354.0	78.0
	3	6	334.2	77.0
	4	24	329.1	76.7

Scrutiny of table IX makes it quite obvious that there is here, with *Martynia* and *Xanthium* as with *Zea* in series IV, a definite relation between the magnitude of the ratio of the calculated to the observed soil moisture residue and the physical nature of the soil mixture. In the present series neither plant shows such inconsistency in the ratios in question as does *Zea* in the preceding series; nevertheless, the range and overlapping are too great here also to warrant any attempt at quantitative interpretation, excepting in the most general way. For such a study many more observations than are here at hand would be requisite. From the results presented, however, it is clearly emphasized that the ratio of calculated to observed soil moisture content at permanent wilting is generally greater the lower the water capacity of the soil.

In connection with this series it is to be remarked that the moisture content of foliage for each of these forms at the time of wilting (table X), shows but slight variations and is apparently not dependent upon the aerial conditions under which permanent loss of turgor occurs. It is suggested by the few determinations here presented that this foliar moisture content at the time of wilting is somewhat greater in the case of *Martynia* than in that of *Xanthium*.

Series VI. The purpose of this series was to determine the effect of repeated wilting in the moist chamber upon the soil moisture residue at permanent wilting, for plants grown in the open but not previously wilted. The six soils used for *Xanthium* in series V were employed. Twelve cylinders of each mixture were prepared, six of each being planted with maize and six with *Phaseolus*. The plants were seven weeks old when sealed and transferred to the moist chamber for the first wilting. They were wilted three times, the second wilting occurring after an interval of eight days, and the third after another one of nine days had been allowed for complete resumption of growth. Throughout this time the plants remained in the moist chamber, in which the temperature ranged from 24.1° to 31.3° C, while the relative humidity varied from 96 to 100 per cent. daily. A summary of the results is given in tables XI and XII.

TABLE XI (series VI)

Ratio of observed to calculated percentage of soil moisture at permanent wilting of *Zea* and *Phaseolus*, repeatedly wilted in moist chamber, the plants being seven weeks old at the beginning of the experiment.

Soil mixture, *per cent.* of sand	*Zea*			*Phaseolus*		
	First wilting	Second wilting	Third wilting	First wilting	Second wilting	Third wilting
100	1.04	1.63	1.64	1.19	2.02	2.58
80	1.14	1.55	1.80	1.13	1.75	2.46
60	1.04	1.33	1.50	1.04	1.67	2.31
40	1.07	1.32	1.42	1.07	1.26	1.57
20	1.02	1.32	1.37	1.02	1.38	1.67
00[37]	1.03	1.28	1.45	1.03	1.34	1.42

[37] Loam without admixture of sand.

From table XI it appears that, in so far as the first wilting is concerned, the soil moisture residues found for both *Zea* and *Phaseolus* are very close to the calculated figures for the soils of higher water-holding capacity, gradually departing from these figures as the percentage of sand in the soil mixture is increased, though the overlapping here seen is considerable. At the second wilting there is, for all the soils, an abrupt and large increase in the amount of residual water, while a still further increase occurs at the third wilting. In both these wiltings, however, the amount of this increase is least in the soil of highest water-holding capacity, namely pure loam, and is augmented in fairly regular fashion with every increase in the percentage of sand in the soil mixture, being greatest in pure sand. Microscopic examination of the roots of these plants showed that the death of root hairs, already shown to accompany wilting in the moist chamber, is of less universal occurrence in pure loam or in mixtures in which loam predominates, than in mixtures having sand to the amount of one-half or more. After the second wilting, no living root-hairs could be found upon plants of *Phaseolus* in soils having 60 per cent. or more of sand, and examination of such plants, after an interval sufficient to allow resumption of growth, showed that the functioning roothairs were sharply confined to the region of the root developed subsequent to wilting, the older portions of such roots showing only hairs whose contents were in various stages of disintegration. For *Zea*, the statements just made do not hold good in their entirety; while it is generally true that there is more general destruction of the roothairs in the soils of lower water-holding capacity, even the plants grown in pure sand show occasional surviving hairs, and in mixtures there is great variation in the degree to which the root hairs of plants from the same soil or even from the same container are destroyed. In spite of considerable variations, it was immediately apparent that the great increase in soil moisture at second or third wiltings, for soils having the lower water-holding capacities, was due, both for *Zea* and for *Phaseolus*, to a very general destruction of the roothairs at the first wilting, and it was equally evident that this destruction became less universal as soils having larger and larger percentages of loam, and consequently higher water-holding capacities, were employed. The ratio of observed to calculated soil moisture residue, at a second permanent wilting under conditions of approximate saturation of the atmosphere, is therefore a fairly accurate indication of the extent to which the root hairs of the plant have been destroyed by the previous treatment of the plant.

The data of table XII again bring out the frequently observed point that the moisture content of the leaves at the time of permanent wilting is nearly a constant quantity. In the present cases there appears to be

TABLE XII (series VI, continued)

Percentage of leaf moisture of plants wilting in moist chamber.

Plant	Wilting No.	Number of plants wilting	Foliar moisture, *per cent.* of	
			Dry wt.	Fresh wt.
	1	6	487	83.[illegible]
Zea	2	8	489	83.0
	3	24	515	83.8
	1	6	434	81.3
Phaseolus	2	16	4[illegible]7	82.4
	3	14	496	83.2

a relatively slight rise in this moisture content with the prolonged exposure to the conditions of the moist chamber and to repeated wilting, but such increased foliar moisture content arising from repeated wilting has nowhere been apparent in the other experiments of these studies and is therefore probably not of general occurrence.

Series VII. In all the experiments thus far considered, the plants employed remained constantly in the open, from germination to the time of their use in the experiments. It may fairly be asked, however, whether the leaf moisture content and that of the soil, found for wilting plants which have been abruptly transferred from the rigorous conditions of the open to the partial shelter or to the moist chamber, are comparable to the corresponding water contents which might be shown by plants grown from the beginning under the latter conditions. The series now to be described was planned to throw light upon this point.

Thirty cylinders were prepared with each of three soil mixtures containing respectively 90, 50, and 10 per cent. of sand, and having calculated wilting coefficients of 3.79, 6.24 and 10.04 per cent. These were designated as groups A, B, and C, and were planted with maize on the same day. The thirty cylinders of each group were divided into three sets of ten each, designated A1, A2, A3, B1, B2, etc. Sets A1, B1, and C1 were placed in the open, A2, B2, and C2 in the lath shelter, and A3, B3, and C3 in the moist chamber. All were given identical treatment in other respects and were allowed to grow until six weeks old. The thirty plants in the open were then transferred to the lath shelter, beside the similar series already there, the entire sixty were sealed, and wilting was allowed to occur in the shelter. Of each of the six lots of ten each, five were taken for leaf and soil samples at the time of wilting, the remaining thirty plants being then transferred to the moist chamber, watered and allowed to recover. The group grown in the moist chamber was then sealed and allowed to wilt together with the remaining members of the two groups which had been grown in the open and in the lath shelter. Tables XIII and XIV summarize the results of this series.

TABLE XIII (series VII)

Soil and leaf moisture contents for *Zea*, the plants six weeks old, grown in open and in lath shelter, and wilted together in shelter.

Group No.	No. of plants	Previously grown in	Soil employed, *per cent.* of sand	Calculated "wilting coefficient"	Ratio of observed to calculated soil moisture residue at permanent wilting	Leaf moisture, *per cent.* of	
						Dry wt.	Fresh wt.
A1	5	Open.......	90	3·79	1.41	486.3	83.0
A2	5	Shelter......	90	3·79	1.39	482.9	81.7
B1	5	Open.......	50	6.24	1.24	478.4	82.7
B2	5	Shelter......	50	6.24	1.27	485.0	83.0
C1	5	Open.......	10	10.04	1.10	483.3	82.9
C2	5	Shelter......	10	10.04	1.13	489.2	83.1

TABLE XIV (series VII, continued)

Soil and leaf moisture content for *Zea*, the plants seven weeks old, grown in open, in lath shelter, and in moist chamber, when wilted together in chamber.

Group No.	No. of plants	Previously grown in	Soil employed, *per cent.* of sand	Calculated "wilting coefficient"	Ratio of observed to calculated soil moisture residue at permanent wilting	Leaf moisture, *per cent.* of	
						Dry wt.	Fresh wt.
A1	5	Open[38]......	90	3·79	1.12	488	83.0
A2	5	Shelter[38].....	90	3·79	1.09	483	82.9
A3	10	Chamber....	90	3·79	1.11	479	82.7
B1	5	Open[38]......	50	6.24	1.08	481	82.8
B2	5	Shelter[38].....	50	6.24	1.05	479	82.7
B3	10	Chamber....	50	6.24	1.04	490	83.0
C1	5	Open[38]......	10	10.04	1.01	483	82.9
C2	5	Shelter[38].....	10	10.04	1.03	476	82.7
C3	10	Chamber....	10	10.04	1.00	487	83.0

Series VIII. It remained to be determined whether the reverse change of conditions to that just considered—transfer from a location of lower to one of higher evaporation rate—would affect the magnitude of the observed soil moisture residue. Series VIII bears upon this point. It was identical with series VII in that the same three soils were used, ten plants being grown in each soil in the open, ten in the lath shelter and ten in the moist chamber. Their treatment, however, was the exact opposite of that given the cultures of series VII; the group grown in the moist chamber was transferred, when six weeks old, to the lath shelter and there wilted together with the group grown in the shelter. Leaf and soil samples were taken as in series VII and the remaining plants were allowed to recover in the moist chamber, after which they were transferred to the open and wilted simultaneously with the group grown there. The results are shown in tables XV and XVI, where the different groups are designated quite as in series VII.

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[38] Previously wilted in shelter and revived, with watering, in chamber.

TABLE XV (series VIII)

Soil and leaf moisture content for *Zea*, the plants six weeks old, grown in moist chamber and in lath shelter, and wilted together in lath shelter.

Group No.	No. of plants	Previously grown in	Soil employed, *per cent.* of sand	Calculated "wilting coefficient"	Ratio of observed to calculated soil moisture residue at permanent wilting	Leaf moisture, *per cent.* of	
						Dry wt.	Fresh wt.
A3	5	Chamber....	90	3.79	1.30	488.6	83.0
A2	5	Shelter......	90	3.79	1.28	475.5	82.6
B3	5	Chamber....	50	6.24	1.19	480.1	82.8
B2	5	Shelter......	50	6.24	1.21	478.2	82.8
C3	5	Chamber....	10	10.04	1.13	474.1	82.6
C2	5	Shelter......	10	10.04	1.16	482.6	82.8

TABLE XVI (series VIII, continued)

Soil and leaf moisture content for *Zea*, the plants seven weeks old, grown in moist chamber, in lath shelter and in open, and wilted together in open.

Group No.	No. of plants	Previously grown in	Soil employed, *per cent.* of sand	Calculated "wilting coefficient"	Ratio of observed to calculated soil moisture residue at permanent wilting	Leaf moisture, *per cent.* of	
						Dry wt.	Fresh wt.
A3	5	Chamber....	90	3.79	1.46	481.6	82.8
A2	5	Shelter......	90	3.79	1.43	473.2	82.6
A1	10	Open........	90	3.79	1.44	478.3	82.7
B3	5	Chamber....	50	6.24	1.28	490.0	83.0
B2	5	Shelter......	50	6.24	1.25	473.6	82.6
B1	10	Open.......	50	6.24	1.23	469.6	82.4
C3	5	Chamber....	10	10.04	1.13	469.8	82.4
C2	5	Shelter......	10	10.04	1.09	473.4	82.6
C1	10	Open.......	10	10.04	1.08	478.8	82.8

While the plants of the last series promptly began to wilt as soon as exposed to the higher evaporation rates, just as was the case with Brown's similarly treated plants ([12], page 130), it was nevertheless possible to determine clearly the point of permanent wilting. The results of this and the preceding series show that transfer from a situation of higher to one of lower evaporation rate, or in the reverse direction, is without appreciable effect either upon the percentage of leaf moisture or upon that of soil moisture remaining when permanent wilting has become established. As regards the soil moisture residues, the agreement of the results from the variously treated groups of plants is close, and the differences are everywhere such as come within the limits of experimental error. From these two series it also appears that the ratios of observed to calculated soil moisture residues show the same variation, in relation to the nature of the soil mixtures, that has been pointed out above; the highest ratios occur with the soil containing 90 per cent. of sand, the lowest with the soil containing 10 per cent., while the ratios occurring with the intermediate soil mixture (50 per cent. sand) are intermediate in value. It is also manifest from series VII and VIII that the amount of moisture in the foliage at the time of wilting is quite unaffected either by the various previous treatments of the plants or by the soil properties.

DISCUSSION.

The results here presented are naturally to be considered under two chief heads, the one concerned with water content of foliage at permanent wilting, the other with the relation of the soil moisture found at wilting, under the various experimental conditions, to the "wilting coefficient" as calculated by the formula of Briggs and Shantz [12, *2,3,4;* 13]. The following discussion of the results obtained from the experimentation already described will therefore proceed under two headings, (1) Foliar moisture content and (2) Soil moisture content at the time of permanent wilting.

(1) Foliar Moisture Content.

Moisture content of leaves at permanent wilting. It appears from the results of determinations of leaf moisture at the time of permanent wilting that we are here dealing with a quantity which is in very high degree constant for any given species. The water present in the leaves at this time is the amount remaining when the supply available to the roots has been so far exhausted that turgor can be restored only by addition of water to the soil. This point is reached many hours after visible wilting has occurred, and with low evaporation rates its appearance may be delayed for a day or more. The only criterion available to the observer in determining whether permanent wilting has been reached, short of actual transfer to a saturated atmosphere, is the degree of flaccidity of the plant, and it is only after scores of observations upon a species have been confirmed by tests in the moist chamber that confident decisions may be rendered.

In every doubtful case the plants have been left for a somewhat longer time before leaf or soil samples have been taken, so that the error thus introduced has tended slightly to lower the averages; no doubt a considerable amount of the apparent variations in these moisture contents, as above presented, may be due to this procedure.

But the variations just mentioned are confined within narrow limits. They are entirely independent of the physical properties of the soil used, of the aerial conditions under which wilting occurs, and of previous wiltings under the same or different conditions; for all the soils and for all three aerial environments here employed the results are practically identical. Furthermore, within the time-limits covered by the experiments, the averages are not affected by the age of the plants, since the general averages for plants six weeks old and for others of the same series but older by from three to five weeks, show no considerable differences (tables III, IV, V, VI, X and XII-XVI). It is obvious, however, that the age factor can be without effect only so long as the formation of new leaves keeps pace with the development of mechanical tissue in the older ones, that is, only through the period of active growth; percentages of water content in terms of dry weight such as are here given are manifestly not applicable to very young plants, with only immature leaves, nor to old plants whose leaves are practically all full grown. For the middle period of the plant's existence, however, it would appear that we have in the percentage of foliar moisture content at the time of permanent wilting a true physiological constant.

Moisture content of leaves at first appearance of wilting. While only secondary attention has been given in these experiments to the matter of foliage moisture content at the onset of wilting, a very considerable mass of data bearing upon this point has incidentally accumulated and may now be briefly noted. The results obtained, which are quite accordant, indicate that the water content of normal functioning leaves at the time of the first appearance of wilting and for any given species during its period of active growth, fluctuates only within narrow limits; in other words, the percentage of water which is just sufficient to maintain turgidity in the functional leaves of a given plant is practically a constant for all plants of that species at approximately the same degree of development. Any further decrease of the amount of water present necessarily results in loss of turgor and in visible wilting, which therefore occurs at a relatively fixed point. Variations from this average wilting point, as shown by table I, are confined to a range of from 4 to 5 per cent. of the fresh weight of the leaves. Excluding errors of observation, these variations are due to such causes as unequal rates of growth and the consequent production of differing proportions of young and old leaves in plants of the same age, or to differences in the rate and degree of development of mechanical tissues in the leaves ("inherent variability" of structure). Obviously such factors as the physical structure of the soil, or the environmental air conditions under which wilting may occur, operate only to reduce the water content of the plant to the wilting point and are without effect upon the magnitude of that content when wilting occurs. Any soil condition (e. g., drying out) which tends to retard water entrance into the roots, or any aerial condition (e. g., increased evaporating power of the air) which tends to accelerate the rate of water loss from the foliage, must, *ceteris paribus*, increase the saturation deficit of the plant as a whole, and so bring the leaves nearer to their wilting point. Long before the occurrence of temporary wilting, however (as appears from the studies of Livingston [11], and of Livingston and Brown [12]), the foliage passes into the condition of incipient drying, and growth is retarded or checked. If, under these conditions, the soil moisture were to be increased (as by watering), the rate of entrance of moisture through the roots should also rise, the further advance of incipient drying should be prevented or retarded, and growth might be resumed without the occurrence of wilting at all. Since it is possible, with many plants at least, to detect incipient drying by determinations of leaf moisture (Livingston and Brown [12], page 330), and to accomplish this long before wilting of any sort becomes manifest, such determination of leaf moisture seems to offer a far more satisfactory indication of the condition of the plant with respect to water than can be hoped for from determinations of soil moisture. This deduction should hold at least in regions where very high rates of evaporation are of occasional or constant occurrence, and such are the very regions where problems of the water relations of plants are most apt to attract attention.

From the data of table I, regarding the water content of the foliage of turgid, wilting and permanently wilted plants, considerable support is given to the idea presented earlier in this paper, that at least three physiologically critical moisture contents may be formulated for plants: (1) an optimum, not sharply defined, in the presence of which turgor is maintained and growth progresses; (2) a temporary wilting content, at which turgor completely disappears; and (3) a permanent wilting content, after the attainment of which recovery is not possible without addition of water to the soil. The last two have been shown above to be fairly definite and constant quantities for several species and under a variety of environmental conditions. Knowledge of these critical water contents should furnish criteria for the further interpretation of the condition of incipient drying mentioned in the last paragraph and, in general, for the determination of the relation of the plant to its water supply and water loss.

(2) SOIL MOISTURE CONTENT AT THE TIME OF PERMANENT WILTING.

The relation of observed to calculated soil moisture residue.

As a result of their very extensive studies on the amount of moisture left in the soil by a large variety of plants wilting under relatively humid conditions, Briggs and Shantz [12, *4*] have reached the following conclusion: "Although the assumption has been made that the wilting point will be proportionately higher in an arid than in a humid region, our results both in the laboratory and in the field indicate that this is not the case" (page 23). "These determinations . . . indicate that the wilting coefficient is not materially influenced by the dryness of the air, by moderate changes in solar intensity, or by differences in the amount of soil moisture available during the period of growth" (page 22). This conclusion rests upon determinations made upon duplicate series, one grown and allowed to wilt in a "dry greenhouse," the other in a moist chamber, and upon similar paired series in the greenhouse, one of which wilted in full sunlight, the other under cheesecloth. In all of these series the observed soil moisture residue at the time of permanent wilting was found to vary in a definite manner with the physical properties of the soil employed, but with any single soil it remained practically constant for a very large number of plants and also throughout the range of aerial conditions employed in the experiments. The very satisfactory agreement among the results of the numerous wilting tests carried out by Briggs and Shantz leaves no room for doubt that their conclusion expresses the truth for the conditions under which they worked. Unfortunately, however, although these authors employ refined methods for determining and stating the physical properties of the soils used and the "wilting coefficients" obtained, yet their statements regarding the aerial conditions for their greenhouse and chamber furnish data for but a very vague and general sort of comparison, not only between the different aerial complexes employed by themselves, but also between these complexes and the conditions employed by other workers.

On *a priori* grounds it seems obvious that conditions of transpiration must be at least theoretically possible for which the physical nature of the soil cannot be the sole factor in the determination of the soil moisture residue at permanent wilting. Without entering into detail in this matter, which will be familiar to students of plant physics, it need only to be remarked here that permanent wilting of the plant occurs with, and depends upon, some critical relation between the three factors, rate of entrance of water from the soil, rate of water movement from roots to foliage, and rate of exit of water from the transpiring surfaces. It should therefore be conceivable for moisture to leave the foliage so rapidly that these parts might attain a stage of drying out from which recovery would be impossible, even though the roots were at the same time in a saturated soil or in water. Whether just this condition may be attained experimentally is still unknown, but some considerations regarding the general nature of permanent wilting will have place later in this discussion. All that needs emphasis here is that failure of a certain amount of variation in the aerial conditions to influence the quantity of residual water left in the soil at permanent wilting, must not be assumed to mean that no possible variation in the aerial complex may exert an influence upon this residuum of soil moisture.[39]

It thus follows that the "wilting coefficient" as established by Briggs and Shantz, and the definite relation which this quantity exhibits toward the physical constants of the soil under the aerial conditions of these authors' experiments, cannot be considered as resting upon a satisfactory foundation without some quantitative knowledge of the range of conditions under which it may be expected to hold. In the absence of definite records and adequate integrations of such aerial factors as temperature, relative humidity, solar intensity and evaporating power of the air, no satisfactory consideration is now possible of the extent of the differences in these conditions which have been contrasted by Briggs and Shantz. Nor is it now possible satisfactorily to compare any of the aerial complexes employed by these authors with those of the open, at any time and place, or with those of any other experimenter. Brown ([12], page 133) has already called attention to these points. The most satisfactory description of the aerial conditions used by Briggs and Shantz must be a statement based on the general climatology of Washington, for the greenhouse experiments, and that of Akron, Colorado, for the field test which they record. The greenhouse tests took place in a "dry greenhouse," with or without cheesecloth shade, or in a "glass damp chamber." The cheesecloth shade "reduced the intensity of direct sunlight to about one-half normal" ([12, *4*], page 23). The time of year at which the different greenhouse tests were performed is not given, so that our knowledge of the climatology of Washington is of little avail in estimating the probable ranges of the atmospheric conditions. For one series, without shade, the temperature of the greenhouse was "about 70° F. and the relative humidity about 85 per cent." ([12, *f*], page 15). No atmometric data are presented for the experiments of these writers.

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[39] After proofsheets of this paper were in the author's hands, the article of Briggs and Shantz on "Application of wilting coefficient determinations in agronomic investigations" (Proceed. Amer. Soc. Agronomy 3: 250-260. 1912) was received. The discussion of this paper by R.O.E. Davis, as summarized in the printed report of the proceedings (page 260), states for the first time the factors entering into the production of wilting with such clearness and completeness that I quote the summary entire. "The point should be emphasized that the wilting of a plant, which is made the basis for the determination of the moisture content below which the plant does not maintain healthy growth, is dependent on many factors. The wilting is due to excess of transpiration over absorption of water. The rate of transpiration depends on temperature, humidity and wind velocity, size of leaves, number of stomata, and the concentration of the cell solution. The rate of absorption is effected by the character of the soil, the concentration of soil solution and of cell solution, the temperature, and the character of the plant roots."

It is at least highly probable that the climatic conditions prevailing in the summer at Tucson include temperatures, light intensities, and evaporating powers of the air much higher than those prevailing in the experiments of Briggs and Shantz. It seems also probable, however, that the aerial conditions of the moist chamber tests at Tucson approached those of the similar tests at Washington, and it was thus to be expected that the results obtained in these two series should be in rather close agreement; indeed, if it were requisite to provide like aerial conditions in the open and during summer at Tucson and in the Washington greenhouse, no more simple and promising method could be devised than that which might employ a shaded moist chamber in both localities. The Washington authors have satisfactorily shown that, within the range of aerial conditions which they employed, these conditions are practically without effect upon the amount of water retained by their soils at the permanent wilting of their plants; their tests gave the same results in the greenhouse without shade (maximum evaporating power of the air) and in the moist chamber (minimum evaporating power of the air). The only change in aerial conditions from those employed at Washington, which could logically be expected to influence the soil moisture content which we are considering, must thus be one resulting in a higher intensity of the water-extracting power of the surroundings than any which Briggs and Shantz employed. Such a higher intensity (practically represented by higher evaporation rate) was surely experienced in the work in the lath shelter and in the open at Tucson, so that it is obvious that the summer conditions at the Desert Laboratory were such as to furnish a somewhat extreme test of the general conclusions of these writers. If aerial conditions exercise an appreciable effect upon the soil moisture content at the time of wilting, this effect should be at its maximum where extreme dryness of the air, almost constant wind, high solar intensity and long sustained high temperatures, produce rates of evaporation several fold greater than those usually reached, even as maxima, in the more humid regions.

The results of the present studies will now be considered in four groups, (a) first wiltings in the moist chamber, (b) repeated wiltings in the moist chamber, (c) wiltings in the open, and (d) wiltings in the lath shelter.

(a) *First wiltings in the moist chamber.* From the experimental data already given for first wiltings in the moist chamber (tables IV, VIII, IX, XI, and XIV), it is clear that, for the less sandy soil mixtures here used, the soil moisture residue at permanent wilting very generally approximate those obtained by calculation from the water capacities of the soils, using the formula of Briggs and Shantz. This seems equally true for all the plant forms here studied, *Zea*, *Martynia*, *Xanthium*, and *Phaseolus*. As long as a plant has not been previously wilted in the moist chamber, it appears to make no difference whether or not it has previously suffered wilting in the open or in the shelter; if the soil be pure or nearly pure loam the ratio of the observed to the calculated soil moisture residues is, in all cases, approximately unity. These ratios rise, however, more or less regularly as the percentage of sand in the soil mixtures is increased, but even in soils of great sand content, the departures of the observed from the calculated "wilting coefficients" are seldom very great. It may be concluded, therefore, that *first* wiltings are very apt to give observed soil moisture residues practically the same as the calculated ones, if the soil possesses a relatively high-water holding capacity, and if the wiltings occur in the nearly saturated, nearly motionless air of the moist chamber, the latter kept at shade temperature but shielded from direct solar insolation.

(b) *Repeated wiltings in the moist chamber.* In all cases of repeated wiltings in the moist chamber the soil moisture contents present at the *second* and *third* wiltings are markedly higher than those shown at the first wilting of the same cultures, hence the observed residues of soil moisture here depart much farther from those calculated for the corresponding soils than do those from the first wiltings in the chamber (see tables VII, VIII and XI), and this departure is much more pronounced for the more sandy soils than for the more loamy ones. The reasons for these discrepancies between the results of the first wilting and those of later wiltings in the chamber have already received some mention, and will be more fully considered after the results from wiltings in the open have been reviewed.

(c) *Wiltings in the open.* When wilting occurs in the open, the ratios of observed to calculated soil moisture residues are generally of much greater magnitude than when wilting occurs in the moist chamber (tables III, IV, VII, IX, and XVII; compare, also, the other tables referring to wiltings in the chamber). It is thus manifest that the formula of Briggs and Shantz [12, *4*] for deriving the water content of a given soil at the permanent wilting of plants rooted therein from its water-holding capacity, is not at all applicable to the wiltings in the open which were included in these studies.

Unlike the conclusions derived from the experiments in the moist chamber, repeated wilting in the open fails to have the effect of raising the value of the soil moisture residue (table IX) for the later wiltings, and, as has been pointed out, wilting in the open or in the shelter is without effect upon the magnitude of the observed residue for a later wilting in the moist chamber.

The extent to which the physical characters of the soil used enter into the departure of the observed from the calculated soil moisture contents at the time of permanent wilting in the open, is clear from tables VII, IX, XV, and XVI. Here the ratio representing this departure undergoes a progressive increase as we pass from pure or nearly pure loam of relatively high water-holding capacity, through a graded loam-sand series to pure sand with a low capacity.

The influence of aerial conditions upon the magnitude of the water content of the soil at the time of wilting in the open appears to be very important, and its consideration will throw light upon the problem as to why the formula of Briggs and Shantz, which has proved itself so satisfactory for wiltings occurring under conditions of low evaporation rate, should fail so completely in the present experiments in the open. Brown ([12], pages 131-134) found that the moisture content of the soil at the time of temporary wilting was fairly constant for any given rate of evaporation prevailing at the time of wilting, and rose in rather irregular fashion, with increase in the evaporation rate, until that rate had attained its maximum for the day. Inasmuch as temporary wilting is merely a visible indication that transpiration has been occurring for some time at a rate exceeding the rate of water supply to the foliage, this is precisely what we should expect. It is not, however, so readily understood why the percentages of soil moisture found at *permanent* wilting should agree in this respect with those for temporary wilting found by Brown, but it became evident early in the present work that such is indeed the case. The moisture contents left in the soil at permanent wilting vary not merely with the large differences in environmental conditions existing in the different places of wilting, but also with the varying evaporation rate in any one place on any single day. The results shown by series I, II, and III bring out this fact very clearly; the use of only one soil in each of these series facilitates comparison, and the range of temperature and humidity is sufficiently great in these cases to be fairly representative of summer conditions at Tucson. From tables III and IV and from the graphs of series II and III (figs. 1-14) it is to be noted that the amounts of water left in the soil by plants reaching the permanently wilted condition in the earlier hours of the day are nearest to the calculated values. As the evaporating power of the air is increased,—by decreasing humidity and rising temperature,—the residual moisture contents rise also in value, until the maximum evaporation rate for the day has been attained and passed, after which they cease to rise or undergo a more or less evident decline. This point may be made more evident by comparing the atmometer reading for each hour of the day at which wilting occurred, with the average of the corresponding ratios of observed to calculated soil moisture content at permanent wilting. The data of series II and III have been thus treated and the resulting quantities are given in tables XVII and XVIII. Three successive wiltings in the open are represented in each series.

TABLE XVII (series II)

Hourly rates of evaporation from the Livingston standard atmometer for the periods of the day during which wilting occurred, together with the average ratio of observed to calculated soil moisture residues, for *Zea* permanently wilting in the open during that period.

First wilting			Second wilting			Third wilting		
Hour	Evaporation, *cc.*	Average ratio	Hour	Evaporation, *cc.*	Average ratio	Hour	Evaporation, *cc.*	Average ratio
8-9	2.8-2.9	1.15	....	...	....	....	...	....
11-12	3.7-3.8	1.37	9-11	3.6-3.9	1.30	7-9	3.7-3.9	1.12
12-13	4.4-4.6	1.67	....	...	....	9-11	4.3-4.4	1.13
.	...	...	11-13	5.4-5.5	1.57	....	...	....
14-15	4.1	1.50	13-14	5.1	1.70	11-13	5.9-6.0	1.34
15-16	3.8	1.75	14-15	3.6	1.81	13-14	5.0	1.43
16-17	2.8	1.89						

TABLE XVIII (series III)

Hourly rates of evaporation from the Livingston standard atmometer for the periods of the day at which wilting occurred, together with the average ratio of observed to calculated soil moisture residues, for *Zea* permanently wilting in the open during that period.

First wilting			Second wilting			Third wilting		
Hour	Evaporation, *cc.*	Average ratio	Hour	Evaporation, *cc.*	Average ratio	Hour	Evaporation, *cc.*	Average ratio
			7:30- 8:30	3.1	1.37	7:30- 8:30	4.5	1.44
			8:30-9:30	5.0	1.50	8:30- 9:30	5.3	1.42
9:30-10:30	5.5-6.0	1.26	9:30-10:30	7.0	1.46	9:30-10:30	7.5	1.43
....	.....	...	10:30-11:30	7.8	1.42	....	...	...
11:30-12:30	6.9-6.6	1.22	11:30-12:30	8.4	1.41	11:30-13:30	8.3-8.6	1.33
12:30-13:30	6.8-7.0	1.20	12:30-13:30	7.0	1.41	....	...	...
13:30-14:30	4.0	1.17	13:30-14:30	5.5	1.40	....	...	....
15:30-16:30	3.6	1.15				14:30-15:30	6.2	1.28

The above data from series II (table XVII) show with especial clearness, as the day advances, a steady rise of observed above calculated soil moisture residues. The maximum evaporation rates attained on the days of these wiltings are not excessive, and prolonged low temperatures with relatively high humidity and absence of wind prevailed during the preceding nights. The plants of this series appear to have been on the verge of the permanently wilted condition at the beginning of each day, and a more or less marked and prolonged increase in the evaporating power of the air (and hence of the transpiration rate) so decreased their contained water that permanent wilting resulted.

The data of series III (table XVIII) show the same phenomenon, though in a somewhat less marked degree. These wiltings occurred in a period marked by high day temperatures and comparatively high humidity at night. The night conditions, by decreasing nocturnal transpiration, appear to have permitted a more complete exhaustion of the soil water before the excessive mid-day transpiration reduced the plants to the permanently wilted condition.

Examination of the results of series IV and V, as well as of those of many similar series not here described, shows quite accordant results for various grades of soil, and leads to the conclusion that the amount of water left in the soil at the time of permanent wilting is not to be generally regarded as independent of the atmospheric conditions under which wilting has occurred. It appears that the value of this water residue for any given soil is determined largely by the evaporating power of the air which obtains between the first appearance of wilting and the attainment of the permanently wilted condition. If this whole period be one of low evaporation rates, so that the rate of transpiration by the plant never greatly exceeds that of its gain by absorption, the wilting condition spreads backward and downward from the leaves until the entire plant body becomes plasmolyzed and the water supply fails at its source. Under these conditions the water content of the whole plant appears to fall with approximately equal rapidity. If, however, the period just mentioned is characterized by high rates of evaporation, the state of permanent wilting may be attained long before water ceases to enter the roots at the usual rate. In such a case the failure of the moisture supply which brings wilting about does not occur at its source, but higher in the plant body, and the residue of soil moisture observed under these conditions must be higher than that observed with low evaporation rates. It appears that, in order that this residue shall be determined solely by soil conditions, it is only necessary that the evaporating power of the air do not surpass a certain limit; it need not closely approach the conditions of the moist chamber, however, for the work of Briggs and Shantz [12, *4*] shows clearly that the amount of soil moisture present at permanent wilting is not appreciably influenced by evaporation rates such as those obtained in the greenhouse at Washington.

It is clear from the foregoing that we cannot consider the plant as a simple indicator, analogous to a piece of physical apparatus, in which a definite condition tells us that the soil about the roots of the plant has reached an equally definite and unvarying condition with respect to its moisture content. The protoplasmic condition denoted by the term *permanent wilting* is brought about through the reduction of the water content of the plant to a rather definite status. Absorption by the roots constantly operates to raise this water content, transpiration to lower it; the relative efficiencies of the two factors determines the time which shall elapse before transpiration gains the mastery, and hence determines also how completely absorption may exhaust the water resources of the soil prior to being overcome. If transpiration be maintained at a rate sufficiently high, loss of turgor will begin in the youngest parts and extend rapidly downward until every part of the plant is involved, even though the soil surrounding the roots contains available water in considerable quantities. If the rate of transpiration be low, the extension of loss of turgor over the plant proceeds more slowly, becoming complete only when absorption by the roots has become insignificant in amount. The amount of water remaining in a given soil after the permanent wilting of plants rooted therein is therefore not a constant for varying sets of environmental conditions, but, beyond a certain limit which has not yet received attention, varies with these conditions. A statement of this soil moisture residuum defines the physical characters of the soil concerned only when it is accompanied by full definition of the aerial conditions, as far as removal of water from the plant is concerned, under which the determination was made. Until the limit has been determined, beyond which the evaporating power of the air cannot pass without beginning to exert an influence on the amount of residual soil moisture at the time of permanent wilting, it will be requisite, in determinations of this residue as a soil character, to maintain a very low rate of evaporation; when the limit just mentioned becomes known it will be only necessary that the evaporation rate do not surpass it.

(d) *Wiltings in the lath shelter.* When the results obtained in wiltings in the lath shelter are compared with those in the open and with those in the moist chamber, it is at once evident that they occupy an intermediate position with respect to the soil moistures found at permanent wilting. The observed residual moisture contents are everywhere in excess of those found in the moist chamber, more closely approaching, but nowhere equalling, those found in the open,—in accordance with the fact that evaporation in the shelter, while never sinking to the merely nominal rate reached in the moist chamber, was always lower than the rates reached outside under corresponding conditions of humidity and temperature. The results from the experiments in the shelter serve, therefore, to illustrate an intermediate condition between that of the chamber and that of the open, for which the general conclusion holds as true as it does for the open.

Condition of root hairs at the time of permanent wilting.

It has been noted that wilting in the moist chamber, followed by later wilting either in the open or in the chamber, shows, at the *second* wilting, soil moisture contents very much greater than those found at the first (tables IX, and X). No such increase in the amount of residual soil moisture is observed when wilting occurs repeatedly in the open or in the partial shelter. This very marked difference is due to the fact that permanent wilting in the moist chamber is accompanied by general death of the root hairs. Microscopic examination of the roots of plants wilted in the chamber reveals a condition of complete plasmolysis of the root hairs, with cessation of protoplasmic movement. Subsequent watering fails to restore turgor or to induce resumption of movement save in very rare and occasional cases. Spalding[40] has described (page 137) a process of regeneration and resumption of growth as occurring in the root hairs of *Verbena ciliata* and *Covillea tridentata* when water was supplied to plants from which it had been withheld for some time, but no evidence of renewed activity or of the presence of life could thus be obtained in root hairs from any of the species wilted in the moist chamber during the present studies. After being watered such plants recover turgor with extreme slowness, manifestly as a result of the great reduction of absorbing surface which accompanied wilting. If again wilted, before time for the development of new root hairs has elapsed, such plants are unable to withdraw any considerable quantity of water from the soil, and hence wilt without regard to the soil moisture content. If well watered and kept in the moist chamber, such plants ultimately develop new root hairs; but if transferred to the open as soon as turgor has been recovered, they die even when their roots are surrounded by almost saturated soil.

In the case of wilting in the moist chamber, we have, as has been pointed out, an evaporation rate so low that absorption from the soil replaces the water lost by transpiration and holds the water content of the plant nearly in equilibrium until the root hairs begin dying, having withdrawn all available water from the soil; after which the water content of the foliage (and of the whole plant as well, as shown by the work of Meschajeff [83]) decreases steadily to the point of permanent wilting. Such plants, if left without water in the moist chamber, slowly withdraw water from the soil by imbibition, as occurred in the cases described by Briggs and Shantz ([12, *4*], pages 16-19), but the amount thus obtained is far too small to permit recovery of turgescence or resumption of growth. The amount of water thus obtained per unit of time will decrease steadily, not only by reason of the drying out of the soil, but also as a result of the progressive reduction of the imbibing surface as disintegration of the root hairs proceeds. While some evidence has been presented, earlier in this paper, which indicates that there are considerable differences in the root hairs of different species as regards their capacity to withstand the effects of such degrees of drying as induce permanent wilting, these differences are not of such magnitude as materially to affect the general result just stated.

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[40] Spalding, Volney M., Biological relations of certain desert shrubs. I. Bot. Gaz. 38: 122-138. 1904.

That no general death of the root hairs occurs in any of the species used, when wilting takes place in the open or in partial shelter, is apparent from the fact that three or four such wiltings may occur without increase in the residual water content of the soil at the time of wilting. Microscopic examination of the roots of plants wilted in the open shows that plasmolysis of the root hairs, while of general and well nigh universal occurrence, is never so pronounced as in the moist chamber wiltings, and protoplasmic movements are more frequently detected in the former case. Such movement becomes generally evident concurrently with the restoration of turgor and resumption of the normal appearance, from 18 to 36 hours after the plant has been watered. That these root hairs continue to grow is evident from the fact that plants examined microscopically at any time subsequent to recovery of turgor, do not show any zone of dying or dead root hairs, but present an appearance identical with that of control plants which have never been wilted. A partial recovery of turgor, in so far as the root hairs are concerned, is of common occurrence when permanently wilted plants are transferred to the moist chamber without addition of water, and it might be expected that the renewed absorption of water thus set up would ultimately restore turgor to the entire plant. This was never seen to occur, however; supposedly "wilted" plants recover within 24 hours after their transfer to the moist chamber, or not at all, unless they are previously watered. While it may be assumed that the reduction of the water content to the point of permanent wilting has here produced some alteration in the protoplasm and consequent reduction in the normal powers of absorption and transfer, such assumption is rendered unnecessary by consideration of the physical facts. The water content of the soil is always considerably below the optimum—near or below the amount at which flagging would appear under minimum evaporation rates as obtained in the open at Tucson—and the rate at which absorption from the soil can occur must therefore be very low. In the absence of the transpiration stream, which has been practically annulled by transfer of the plant to a saturated atmosphere, movement of the absorbed water upward into the plant must occur only by the slow process of cell-to-cell diffusion. There is, therefore, an accumulation of water in the absorbing organs. If now water be added to the soil so that absorption may freely occur, lowering of the osmotic pressures existing in the root hairs results, and the consequent onward diffusion of water ultimately restores turgor to the entire plant. It was found in the present work that removal of a partially recovered plant from the moist chamber to the open, for an hour or two in the late afternoon, considerably hastens recovery, apparently by starting up the transpiration stream and thus facilitating upward distribution of water from the turgescent roots.

Mention has previously been made of the fact that the five species here used differed considerably among themselves with respect to the capacity of their root hairs to recover functional activity after plasmolysis resulting from wilting under conditions producing a low rate of evaporation. This difference is particularly well shown for *Zea* and *Phaseolus* by the results of series VI (table XI) and the less pronounced difference between *Xanthium* and *Martynia* is seen in series V (table IX). Other experiments not here described gave considerable further evidence pointing to specific differences between these forms as regards the readiness with which the root-hairs are destroyed by wilting. Since the ability of its root hairs to recover from the effects of desiccation is manifestly one of the chief factors which determine a given plant's ability to recover from the effects of severe drought, any specific differences which cultivated forms may display becomes a matter of considerable practical as well as theoretical importance, and this point is therefore reserved for further investigation.

GENERAL CONCLUSION.

Permanent wilting appears to be a condition of general plasmolysis (but not of death) in all the tissues of the plant, with accompanying cessation of certain of the protoplasmic activities. This condition is characterized by a water content of the functioning foliage which is nearly constant for any species. The reduction of the water content to this point is the resultant of the action of transpiration versus root absorption; if aerial conditions permit these two processes to go on at approximately equal and moderate rates until the water supply fails at its source, there remains in the soil a quantity definitely related to the physical constants of the soil—the "wilting coefficient" of Briggs and Shantz [12, *4*]. If transpiration be long maintained at a rate considerably exceeding absorption, permanent wilting is attained irrespective of the presence in the soil of more than the theoretical quantity of water which corresponds to the "wilting coefficient" of these authors. This excess of soil water is only effective temporarily to preserve the life of the root hairs; its amount is more or less definitely related to the aerial conditions prevailing throughout the period of wilting and rises with increase in the transpiration rate.

Thus the only constant with which we have to deal in cases of wilting under widely varying conditions is a physiological constant, a condition of the plant protoplasm with respect to water; which arises as a result of the interaction upon the plant of a complex of external conditions, of which the soil moisture content is only one. The soil moisture content at permanent wilting is therefore a variable which increases with change in the value of the other terms of the equation, especially with increase in the evaporating power of the air beyond a certain limit; and it is not to be considered as independent of these other terms. In this respect the conclusion of Briggs and Shantz that the soil moisture residuum here considered is constant under a wide range of aerial conditions, must obviously be modified. The relatively constant moisture contents of the soil at permanent wilting that were obtained by these authors are precisely what should be expected for aerial conditions such as they describe as prevailing in the greenhouse where their experiments were carried on. Equally constant but quite different and higher values should be obtained wherever plants are wilted under conditions which maintain a relatively uniform but considerably higher rate of transpiration than prevailed in their experiments. It is only when the results of experiments made under widely varying conditions are compared that the relation here pointed out, of the residual soil moisture to the aerial environmental complex, becomes discoverable.

That we have in the water content of the soil at the time of permanent wilting, when determined under adequately defined conditions of evaporation, a function destined to be of very great value to plant physiology and to scientific agriculture is beyond question. It is scarcely less certain, however, that the use of a living plant as an indicator of soil conditions must lead to very considerable error unless the experimentation be carried out with low evaporation rates, or accompanied by adequate determination and statement of the aerial conditions. In the wilting of the plant we are dealing, not with a simple physical phenomenon but with a complex physiological problem, a change of condition in living protoplasm. The properties of the soil in which the plant is rooted constitute merely one of the group of environmental factors which collectively operate to bring about this change.

The conditions under which the work here described was done, together with the limited time at my disposal, rendered impossible any detailed study or quantitative measurements of the independent effects of such factors as solar intensity, humidity, air temperature or soil temperature. For the same reason, detailed histological study of the changes in root hairs during the period of wilting were necessarily postponed.

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THE INFLUENCE OF CALCIUM, MAGNESIUM AND POTASSIUM NITRATES UPON THE TOXICITY OF CERTAIN HEAVY METALS TOWARD FUNGUS SPORES.[1]

Lon A. Hawkins.

ABSTRACT.[2]

This study has to do with the influence of one salt in altering the toxic effect of another upon fungus spores. It is shown that the toxic effect of copper nitrate on the germination of Gloeosporium spores can be inhibited or modified by the addition to the medium of calcium nitrate and that the molecular ratio of the quantity of the calcium salt thus required to the amount of copper nitrate present increases with the concentration of the latter. This effect is apparently the result of a simultaneous action of the two salts upon the organism and cannot, in the cases here considered, be related either to formation of an undissociated double salt or to depression of the ionization of the toxic salt because of the ion common to the two salts. Potassium nitrate is also effective in inhibiting or modifying the toxicity of copper nitrate. The influence of calcium upon the toxicity of copper is of interest in connection with the problem of fungicides and fungicidal action.

The toxicity of lead nitrate is similarly influenced by the presence of calcium nitrate, and the molecular proportions in which these two salts may be combined so as just to counteract the toxicity of the former were found to be the same for the three different concentrations of lead nitrate that were employed. The toxicity of this lead salt is likewise influenced by proper concentrations of magnesium nitrate. Both calcium nitrate and magnesium nitrate markedly decrease the toxicity of zinc nitrate, but neither exhibits any effect on the toxicity of aluminum nitrate in the concentrations used in these experiments.

The effects produced by the various single salts upon the germinating spores are of interest. Four types of response to the salt solution are clearly discernible. (1) The effect of the solution may be the same as that of distilled water, the spores germinating normally, with long, narrow tubes. (2) A somewhat higher concentration of the toxic substance may

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allow germination, this being abnormal, however, with the production of unusual and characteristic structures. (3) Germination may be inhibited for eighteen hours, the spores germinating when subsequently transferred from the toxic solution to distilled water. (4) The spores may be killed in eighteen hours and thus be incapable of renewed activity even when transferred to water. It was usually possible to bring about these four types of response by means of any one of the compounds used in this study by a proper choice of concentration. An arrangement of these substances in the order of their toxicity, as evidenced by the inhibition of spore germination, is found to hold also for their effectiveness in bringing about the other and less final changes which lead to abnormal growth. A list of the substances employed, arranged in the order of their toxicity follows: $Cu(NO_3)_2$, $CuSO_4$, $Pb(NO_3)_2$, $Al(NO_3)_3$, HNO_3, $Zn(NO_3)_2$, $Ni(NO_3)_2$, $Mg(NO_3)_2$, $Ca(NO_3)_2$, KNO_3, sucrose.

It is shown that no one of the several hypotheses heretofore proposed to account for the dynamics of the antagonistic salt action is adequate to explain all the facts brought out in this study.

INTRODUCTION.

Numerous instances have been recorded of the influence of salts on the toxicity exerted by various substances upon organisms. This antagonistic action, as it is frequently called, of a salt upon a toxic substance, is of considerable importance in influencing the behavior of organisms in a given environment, or indeed in determining whether or not they may exist at all in certain environments. For example, Loew[3] has shown that the toxic effect on Spirogyra of a one per cent. solution of magnesium nitrate is inhibited by the presence in the medium of a 0.3 per cent. solution of calcium nitrate, while Loeb[4] has demonstrated that the addition of a small quantity of a calcium salt to a 0.625m solution of NaCl inhibits the toxic effect of the NaCl on the development of Fundulus eggs. Osterhout[5] has shown that a physiologically balanced solution, containing sodium chloride, magnesium chloride, magnesium sulphate, potassium chloride and calcium chloride, is necessary for the best growth of certain marine algae. True and Bartlett[6] have brought out the fact that a ratio of one molecule of calcium to nine molecules of magnesium inhibits the toxic effect of rather high concentrations of magnesium upon roots of Canada field peas. Other cases of antagonistic salt action in combinations of salts of the alkali metals or of the alkaline earths have been demonstrated, and some information has been obtained regarding the influence of these salts on the effect of the heavy metals, which seem to be universally toxic. The latter have not received much attention, however, and it seemed worth while to investigate some of these, alone and in the presence of calcium and magnesium, to obtain evidence as to whether the lighter metals may modify in any way the toxicity of the heavy ones. The investigation described in this paper was accordingly undertaken. The problem here involved will be taken up more in detail after some of the literature pertinent to the subject has been considered.

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[5] Osterhout, W. J. V., On the importance of physiologically balanced solutions for plants. I. Marine plants. Bot. Gaz. **42**: 127-134. 1906.

[6] True, R. H., and Bartlett, H. H., Absorption and excretion of salts as influenced by concentration and composition of culture solutions. U.S. Dept. Agric. Bur. Plant. Ind. Bulletin 231. 1912.

One of the earlier studies of the influence of chemical compounds upon the toxicity of the heavy metals was carried out by Krönig and Paul.[7] These authors determined the effects of various heavy metals in combination with many salts as well as with certain acids and bases. Mainly from work with Bacillus anthracis, they regard the influence of other halogen compounds upon the toxicity of mercuric chloride as probably due to a depression of the ionization of the latter salt. In this connection they say (page 110): "Die Desinfectionswirkung wässeriger Mercurichloridlösungen werden durch Zusatz von Halogenverbindungen der Metalle und von Salzäure herabgesetzt. Es ist wahrscheinlich, dass diese Verminderung der Desinfectionskraft auf einer Rückdrängung der elektrolytischen Dissociation beruht."

Clark[8] carefully studied the influence of various concentrations of sodium chloride upon the toxicity of mercuric chloride as regards the process of germination in various fungus spores and seeds and that of growth in yeasts and bacteria. He found that the toxicity of a mercuric chloride solution increased with addition of small quantities of sodium chloride but decreased when high concentrations of the sodium salt were used. He explained these phenomena by considering that a double salt of sodium chloride and mercuric chloride was formed, such as Na_2HgCl_4 or some similar combination. He supposed that the dissociation tension of this double salt was probably much higher than that of mercuric chloride, a consideration which might account for the increased toxicity of combinations in which small amounts of sodium chloride were employed. In this connection, he suggested that the $HgCl_4$ ion present when such a salt as Na_2HgCl_4 dissociated at lower concentrations, might be considerably more toxic than the $HgCl_2$ molecule to which he seems to attribute the toxicity of mercuric chloride solution.

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[7] Krönig, B., and Paul, Th., Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfection. Zeitsch. Hygiene und Infectionskrankheiten **25**: 1-112. 1897.

[8] Clark, J. F., On the toxic value of mercuric chloride and its double salts. Jour. Physic. Chem. **5**: 289-316. 1901.

In a later investigation, on the toxicity of copper in combination with various chemical compounds, the same writer[9] has shown, for spore germination in *Oedocephalum albidum* and *Rhizopus nigricans* that ammonium nitrate, sodium sulphate, potassium sulphate, and potassium chloride all markedly decreased the toxic effects of both copper chloride and copper sulphate. He used relatively high concentrations, in some cases five per cent., of the alkali and ammonium salts. He considers the decreased toxicity just mentioned as probably due to the formation of double salts, as he suggested in the case of mercuric chloride.

Le Renard[10] studied the comparative toxicities of salts of many of the heavy metals upon Penicillium, the fungus being grown in various concentrations of nutrient media. He used the acetates of potassium, magnesium and ammonium alone, and the acetates, formates, nitrates, phosphates, and sulphates of these three metals with glucose. Also, in combination with various concentrations of the salts in the nutrient medium he used several concentrations of the chlorides and nitrates of zinc, nickel, cobalt and copper, mercuric chloride, silver nitrate, and the sulphate and acetate of copper. The presence in the nutrient medium of the lighter metals in higher concentration was usually found to decrease the toxicity of the heavy metals.

True and Gies[11] showed that calcium modified the toxicity of various copper salts, of zinc sulphate and of mercuric chloride, in their effect upon the growth of roots of *Lupinus albus*. In discussing their results these authors say: "The stimulating action of the calcium seems to have operated against the retarding action of the copper, and the result is a marked diminution in the poisonous action of the copper." They thus relate this influence of calcium upon copper to a mutual effect of the two salts on the protoplasm.

Szücs[12] has recently shown that the toxic effect of copper sulphate on the roots of *Cucurbita pepo* may be inhibited by aluminum chloride in certain concentrations. In this case he used as index of toxicity the ability of the root to react to a geotropic stimulus after it had been removed from the poisonous solution. He varied the presentation time of the toxic stimulus (solution of copper sulphate) both alone and with addition of aluminum chloride, and found, for the shorter time periods, that the presence of aluminum inhibited the poisonous action of copper. However, when such a combination of the two salts was allowed to act for longer periods, it also was toxic in many cases, and the roots lost their ability to respond afterwards to the geotropic stimulus. He used concentrations of copper sulphate varying from 0.001875n to 0.075n, combined with aluminum chloride in concentrations varying from 0.005n to 0.45n. The presentation time of the toxic stimulus ranged from 33 minutes to 26 hours and 50 minutes. This writer also studied the effect upon Spirogyra of quinine hydrochloride, methyl violet and piperidine in combination with various other substances. The toxicity of quinine hydrochloride was altered by various concentrations of other substances, being almost inhibited by aluminum nitrate, markedly decreased by calcium nitrate, and only slightly lessened by potassium nitrate. Thus, the effectiveness of these salts in reducing the toxicity of quinine hydrochloride diminished with the valency of the cation. Similar results were obtained with the same series of salts in combination with methyl violet. The effect of combinations of various substances with piperidine was, in most cases, markedly to increase its toxicity. Szücs apparently considers the antagonistic action, as investigated by him, to be due to the lowering of the rate of absorption of the toxic ion by the presence in the solution of another ion of similar sign. He concludes, in summarizing: "Die Ursache der ' antagonistischen Ionenwirkungen ' liegt in allen von mir untersuchten Fällen in der gegenseitigen Beeinflussung der Aufnahmegeschwindigkeit zweier im gleichen Sinne geladener Ionen."

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[9] Clark, J.F., On the toxic properties of some copper compounds with special reference to Bordeaux mixture. Bot. Gaz. **33**: 26-48. 1902.

[10] Le Renard, Alf., Influence du milieu sur la résistance du Pénicille crustacé aux substances toxiques. Ann. Sci. Nat. Bot. IX. **16**: 276-336. 1912.

[11] True, Rodney, H., and Gies, W. J., On the physiological action of some of the heavy metals in mixed solutions. Bull. Torr. Bot. Club **30**: 390-402. 1903.

[12] Szücs, Joseph, Experimentelle Beiträge zu einer Theorie der antagonistischen Ionenwirkungen. Jahrb. wiss. Bot. **52**: 85-143. 1912.

From the results obtained in the investigations just considered, it is apparent that the toxic effect of the heavy metals on an organism can be modified, in some cases at least, by the addition of certain salts in proper concentration. True and Gies, and Szücs, working with higher plants, attribute this influence of one salt on the effect of another to a simultaneous action of the two salts upon the organism itself, while Clark, working with fungi, relates the inhibition of the toxic effect of heavy metals in combination, to some modification of the salts in the solution. The proportions of salts used in the investigations just mentioned were widely different, and it is of course possible that the different conclusions reached may have been due to this feature. Furthermore, as fungi and higher plants so frequently react differently to the same stimulus, it is possible that one of these two explanations might hold for one group of organisms and the other for the other group. The present study, in which a fungus was employed, was undertaken partly to throw light on the question just suggested.

It was the purpose of this research to examine the effects of the nitrates of copper, lead, zinc, nickel, and aluminum, upon the germination of fungus spores, the salts of these heavy metals being used both alone and in combination with the nitrates of calcium and magnesium, to see whether the presence of the lighter metals in various concentrations might or might not decrease the toxic effect of the heavy ones. It was also considered worth while, when such a decrease was found to occur, to determine as far as possible whether this influence might be related to a direct effect of salts on each other in the solution or to some modification of the organism itself. Furthermore the results obtained in these experiments should throw some light on the problem of the comparative toxicity of the various substances here employed, when used alone, and thus upon the general physiological problem of toxicity.

The investigation was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, and the writer's sincere thanks are due to Professor Burton E. Livingston for his many helpful suggestions and valued assistance throughout the progress of the work.

ORGANISM.

The fungus spores used in this research were of the Gloeosporium or conidial stage of *Glomerella cingulata* (Stonem.) S. and v. S., the fungus causing the disease of the apple known as "bitter rot." Not only is the fungus parasitic upon the apple, but according to Shear and Wood,[13] it is also the cause of disease on other plants. On the apple fruit it produces brown, sunken areas, usually nearly circular in shape, which may be covered with the fruiting bodies of the fungus, the conidia being borne in acervuli. In mass, the spores appear orange-colored but have a hyaline appearance under the microscope. They are usually ovate or oblong in shape, the two diameters being 12-16 μ and 4-6 μ. Shear and Wood have shown that certain strains of this fungus, when grown on proper artificial media, may produce conidia for generation after generation, without the interpolation of the ascogenous form at any time. Cultures of such a strain were secured from Dr. Shear for these experiments, and conidia only were produced throughout the investigation, which lasted about eighteen months, though forty or more generations must have passed.

Corn meal agar was used as a medium for the stock cultures. This was prepared by adding 4 teaspoonsful of white corn meal to one litre of distilled water, which was then allowed to stand at about 58° C. for one hour. After filtration, 12.5 g. of agar flour was added to the infusion, and the mixture was steamed for one and one-half hours. It was then re-filtered and was ready to tube and sterilize. The tubes were slanted and the stock cultures were inoculated in streaks. On this medium the fungus produces but little mycelium, at or beneath the surface, and the conidia are borne in relatively large, orange-colored masses on the surface of the medium along the streak. The acervuli are visible in from two to five days after inoculation.

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[13] Shear, C. L., and Wood, Anna K., Studies of fungus parasites belonging to the genus *Glomerella.* U.S. Dept. Agric. Bur. Plant Ind. Bulletin 252. 1913.

Before the spores were used in the experiments, the stock cultures were allowed to grow undisturbed from ten to fifteen days, a procedure which insured a sufficient quantity of spores from a single tube for the inoculation of an entire series of experimental cultures. Preliminary tests indicated that spores from acervuli in the same tube, but of different ages, were not at all uniform in viability. Direct inoculation of the culture dishes from the spore masses of the stock tubes was thus shown to be unsatisfactory, since it was not only desirable that all cultures contain approximately the same number of spores, but also that the percentage of viability of the spores in all cultures should be as nearly alike as possible. It also seemed desirable to avoid small pieces of agar and bits of mycelium in the liquid cultures, for such contamination might influence the effect of the salts in the solutions on the germination of the spores, as by absorption or the formation of new chemical compounds. The plan was therefore evolved of allowing the stock cultures to grow for from ten to fifteen days, after which period the spore masses were carefully removed, with a platinum needle, to a clean area on the agar surface, from which, after thorough mixing in a little heap, the inoculations to the water cultures were made. This method usually resulted in satisfactory uniformity in the germination of the spores in the various controls, from which fact it was concluded that all cultures thus made contained an approximately equal number of viable spores, and that any inhibition or modification of germination must be due to properties of the culture medium rather than to differences in the spores introduced.

In comparing the effect of the various media upon the spores, the main criterion was the presence or absence of germination after a period of eighteen hours. In many cases, however, germination was more or less modified, as in the production of swollen tubes and other abnormalities, and such modifications of germinal activity needed frequently to be taken into account. As has been indicated, it was seldom necessary to consider the percentage of normal germination which occurred, but in many cases the proportion of abnormal to normal growth was approximately determined.

These Gloeosporium spores possess several very favorable features for such an investigation as the present. They are readily wetted by water and aqueous solutions and, being slightly heavier than water, they sink quickly to the bottom of a hanging drop. They germinate readily in distilled water in from three to four hours and produce long filaments in eighteen hours, a feature which is of considerable advantage here, for it is quite conceivable that the influence of various chemical compounds on each other and on the germinating spores might be considerably altered if nutrient salts were present in the solution. In view of these considerations these experiments were carried out without the use of nutrient media.

METHODS.

The salts used in these experiments were " Baker's analysed " chemicals, procured in the original packages. Stock solutions of the different salts, from which the experimental solutions were afterwards prepared, were made up in 0.2m,[14] 0.5m, or molecular concentration. In preparing the stock solutions the salts were weighed in glass-stoppered weighing bottles directly from freshly opened packages and were dissolved in volumetric flasks. These solutions were then made up to the required volume at a temperature of 15° C. They were stored in Jena glass bottles which had been carefully washed with saturated solution of potassium dichromate in sulphuric acid, steamed for half a day, again washed with distilled water and finally allowed to soak in distilled water for a month or more, to remove any soluble matter which might be present. Distilled water from a still with tin lined boiler and condenser was used in making the stock solutions as well as in diluting them for the cultural work. Preliminary tests showed that the spores germinated as well in water from this still as in more nearly pure water distilled from potassium permanganate solution, using a hard glass flask and a block tin condenser.

The stock salt solutions were diluted to the concentrations required in making up the solutions for the experiments, by pipetting out the proper amount into a hard glass beaker and then adding the necessary distilled water from a burette. The concentrations of these solutions were so calculated that the culture solutions could be prepared without the measurement of less than 0.5 cc. in any case. Thus, errors that might have arisen in attempting to read hundredths of a cubic centimeter on a burette graduated only to tenths, were obviated.

In making up a series of cultures, the two salt solutions which were to be combined were separately diluted to twice the concentration finally desired, and were then placed in burettes. From these were prepared, with addition of water as needed, the combinations and concentrations actually used in the experiments. These mixtures, in volumes of 10 cc. or more, were made in small flasks (of about 75 cc. capacity), a flask being provided for each of the different combinations as well as one for the control. The latter solution usually contained the salt of the heavy metal alone.

From each of the flasks just mentioned a small portion of solution (about a cubic centimeter) was placed in a separate glass cylinder (2 cm. high and 3 cm. in diameter), to which spores from a stock culture were then transferred. These inoculations were made in order, beginning with the weakest solution of the lighter metal. A platinum needle was used for this purpose, flamed and washed to sterilize and clean it after each inoculation.

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[14] The letter *m* is used throughout this paper to denote *molecular*, a concentration of a single gram molecule in a liter of solution. There seems to be considerable confusion in this important matter of defining solutions, some chemists employing the letter *n* to denote molecular, although the latter has long been used to denote *chemically equivalent*.

In each case the tip of the needle was dipped a single time in the well mixed mass of spores which had been prepared on the agar surface, as already described, and the spores that adhered were washed off in the culture solution. Thus, approximately the same number of spores were inoculated into all of the glass dishes and the solutions were then ready for the preparation of the hanging drops.

The drop cultures were made after much the same method as that described by Clark.[15] Van Tieghem cells were used, small cylinders of glass tubing, with ground ends, 9 mm. high and 12 mm. in diameter, which were cemented to ordinary microscope slides by means of beeswax. Two cells were affixed to each slide. The culture solutions in the glass dishes, into which spores had already been inoculated, were thoroughly mixed with a glass rod. By means of this rod, a drop of the liquid was then placed upon a flamed cover glass.

A small drop of the culture solution from the corresponding flask without spores was placed in the bottom of the Van Tieghem cell and the cover bearing the drop culture was inverted over it. Duplicate drop cultures were made from each concentration of solution, both cultures being placed on the same slide. As has been shown by Clark, the presence at the bottom of the culture cell of a small amount of the same solution as that from which the hanging drop is composed prevents evaporation from the drop and hence obviates marked alteration in its concentration, even if the cultures remain in the thermostat for a considerable time. Without this precaution the solution contained in the hanging drop is apt to become markedly more concentrated during the period of an experiment, which might lead to erratic results. The covers were sealed in position with petrolatum. It was not found necessary to take the precaution recommended by Clark, of first allowing the expanding air to escape through a small opening in the seal, possibly because the temperature of the thermostat here used was only a little above the temperature at which the preparations were made.

For ease in handling the cultures, the slides were placed in sheet metal trays, which could be piled one upon another in the thermostat so as to form a rack. These trays were 15 cm. wide and 20 cm. long, with vertical flanges at the ends, the flanges extending upward about 1.3 cm. and downward about 0.4 cm. They were so bent that the lower flanges of one tray fitted outside the upper flanges of the next lower one, and many trays could thus be arranged in a compact and rigid pile without any disturbance to the slides. The bottom of each tray was perforated with circular openings about 1.5 cm. in diameter and 0.5 cm. apart, to facilitate circulation of air. Each tray carried fourteen slides. The slides were always transferred to and from the thermostat by means of the trays, a whole series of cultures being thus moved together.

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[15] Clark, J. F., On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**: 289-327. 378-404. 1899.

The cultures were kept during germination in an electrically heated and automatically regulated thermostat, in which the temperature was maintained at or near 25° C. As the temperature of the room in which the thermostat was placed sometimes rose above 25°, it was necessary to install apparatus for absorbing heat at such times. To accomplish this, several coils of thin-walled copper tubing carrying a continuous stream of tap water were placed at the top of the thermostat, surrounding a small motor-driven fan, the latter insuring air circulation. The air of the chamber then tended to assume a temperature several degrees below that of the laboratory, and the thermostat acted as though standing in a cold room.

EXPERIMENTATION.

In these studies, any renewed activity in the protoplasm of the spore was considered as germination. Several forms of such renewed activity are exhibited by the conidia here employed. Without any alteration in size or shape, a portion of the spore may become nearly or quite opaque, thus appearing dark brown or black by transmitted light. A papilla may be formed at any point on the surface. Such papillae may or may not enlarge to form rounded bodies, and may either remain hyaline or become apparently darkened. Papillae may enlarge to form irregularly shaped bodies or may extend outward as markedly thickened tubes. Lastly, the growing papillae may take the form of slender tubes. The latter type of germination always occurs in distilled water and the tubes attain, in a period of eighteen hours, a length at least twice as great as that of the spore itself. In the following treatment germination will be considered "normal" wherever tubes of the last mentioned type, at least twice the length of the spore, were produced in eighteen hours.

In the present section will be considered the various effects upon germination brought about in the presence of the following salts either alone or in certain combinations:

$Ca(NO_3)_2$
$Mg(NO_3)_2$
KNO_3
$Pb(NO_3)_2$
$Zn(NO_3)_2$
$Al(NO_3)_3$
$Ni(NO_3)_2$
$Cu(NO_3)_2$
$CuSO_4$

TOXICITY OF SALT COMBINATIONS

Calcium, Magnesium and Potassium.

It requires a relatively high concentration of calcium, magnesium or potassium nitrate to inhibit the germination of these spores. With $Ca(NO_3)_2$ normal germination was found in the 0.5m solution and swollen tubes were abundant in 0.6m. At a concentration of 0.7m, however, none of the spores germinated in any of the four series of duplicate cultures which were carried out. The concentration of this salt which just prevents germination appears, therefore, to lie between 0.6m and 0.7m. Magnesium nitrate is similar to that of calcium in its effect. With this salt the spores germinated normally at a concentration of 0.4m, and local swellings of the spore wall and swollen tubes were produced in 0.5m solution, while no germination was evident when a 0.6m concentration was employed. The concentration at which $Mg(NO_3)_2$ just inhibited germination thus seems to lie between 0.5m and 0.6m. In solutions of KNO_3 the spores germinated normally with a concentration of 0.9m and formed local swellings of the spore wall with molecular concentration. As the latter was the highest concentration here used, the point at which KNO_3 inhibits germination was not determined.

The exceedingly high concentrations of these salts, which were found necessary to inhibit germination, made it seem possible that they might be without toxic effect upon the spores; the inhibition of germination which was observed in high concentrations might have been the result of the high osmotic pressure exerted by the medium. To obtain evidence on this point the germination of the spores was tested in cane sugar solution at various concentrations. A 0.2m stock solution was prepared from granulated sugar which had been previously pulverized and desiccated; the different concentrations used in the cultures were prepared from this. The spores germinated normally in concentrations of cane sugar up to and including 1.4m, considerable germination in the form of local swellings (shown in fig. 1) was found in the 1.6m solution, and no germination occurred with 1.8m concentration. The concentration of cane sugar which just inhibits germination lies then between 1.6m and 1.8m.

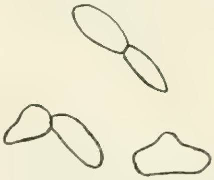

Fig. 1. Germinating spores from 1.6m sucrose solution, × 650.

It will be observed that a 1.6m solution of sucrose has about the same effect upon the germination of these Gloeosporium spores as do 0.6m, 0.5m and molecular solutions of calcium, magnesium and potassium nitrates respectively. Calculations[16] of the osmotic pressures of these four solutions give 51.36, 29.13, 26.00 and 39.31 atmospheres, respectively, from which numbers it becomes obvious that the osmotic concentration of the sugar solution was much greater than that of the other three. It is therefore suggested that, unless the wall and protoplasm of the spores here employed be readily permeable to cane sugar (which seems highly improbable), the inhibiting effects observed with the salt solutions are not to be related primarily to their osmotic properties.

The relatively high osmotic pressure existing within the spores here dealt with, as indicated by the data just given, renders it unnecessary to consider osmotic pressure as a factor in bringing about the modifications and inhibitions of germination which are next to be considered; the concentrations of the various salt solutions employed were always far too low to produce any removal of water from the cells. Similarly, the toxicity of calcium, magnesium and potassium nitrates is so very slight as not to require any consideration in connection with the combinations of these salts with those of the heavy metals; in these combinations the salts of the lighter metals have not here been used in concentrations higher than 0.1m.

The influence of the nitrates of calcium, magnesium and potassium upon the toxicity of salts of the heavy metals will now receive attention.

Copper.

As was to be expected, the effect of $Cu(NO_3)_2$ upon the germination of these spores was found to be widely different from that of $Ca(NO_3)_2$. No germination was ever found in concentrations of the copper salt alone higher than 0.00006m, and it was only in an occasional culture that one or two spores were observed producing local swellings with that concentration. Local swellings of the spore walls and swollen tubes were frequently found with 0.00004m and with 0.000026m concentration, the next lower one which was here used; with 0.00002m solution of $Cu(NO_3)_2$ germination was about as in distilled water.

Copper nitrate was combined with $Ca(NO_3)_2$, at several concentrations of the copper salt well above that at which the latter was non-toxic when used alone. In a series of combinations of 0.0001m $Cu(NO_3)_2$ with different concentrations of the calcium salt, ranging from 0.00003125m to 0.000625m, the spores germinated readily in those cultures where the concentration of $Ca(NO_3)_2$ was 0.0000625m or higher. With the last named concentration many local swellings were found, and also short swollen tubes (see fig. 2), much as in the 0.00004m concentration of $Cu(NO_3)_2$ alone. With the next lower concentration of the calcium salt, 0.00003125m, no germination occurred.

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[16] Morse, H. N., and Holland, W. W. The osmotic pressure of cane sugar solutions at 25°. Amer. Chem. Jour. 41: 1-19. 1909. The osmotic pressure of molecular cane sugar was measured directly by these writers and the osmotic pressure of 1.6 m. cane sugar as given above has been calculated from their tables. The result is probably low, for they found that the ratio of observed to calculated osmotic pressure increases with the concentration. For the gas pressure formula, by which the osmotic pressure of the two electrolytes is calculated, see Landolt, H., Bornstein, R., and Meyerhoffer, W., Physikalische-chemische Tabellen, 3te Auflage. Berlin. 1905; page 24.

From these facts it appears that the toxicity of 0.0001m $Cu(NO_3)_2$ solution is so reduced as to be physiologically equivalent to a 0.00004m solution of the same salt, by the addition to the former solution of five molecules of $Ca(NO_3)_2$ for every eight molecules of $Cu(NO_3)_2$ present. The addition of the lighter metal in this proportion produces the same effect as though the 0.0001m copper solution had been diluted to two and a half times its original volume.

Copper sulphate exhibits about the same toxicity toward these Gloeosporium spores as does the nitrate. A series of combinations of this salt with $Ca(NO_3)_2$, quite similar to the series with $Cu(NO_3)_2$ just described, gave no germination in solutions containing 0.00003125m concentrations of the calcium salt, while germination occurred in some of the cultures containing 0.0000625m $Ca(NO_3)_2$. In general, the effect of $CuSO_4$, either alone or in combination with $Ca(NO_3)_2$, was practically the same as was that of $Cu(NO_3)_2$. This furnishes some additional evidence toward the already rather firmly established conclusion, that the toxicity of copper salts is due to the cations, and it also indicates the probability that considerations bearing upon this toxicity need deal only with the cations.

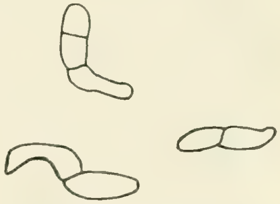

Fig. 2. Germinating spores from combination solution containing 0.00006m $Ca(NO_3)_2$ and 0.0001m $Cu(NO_3)_2$, ×650.

A series of experiments was carried out using 0.0004m $Cu(NO_3)_2$ in combination with concentrations of $Ca(NO_3)_2$ ranging from 0.00025m to 0.025m. The spores in these combinations germinated readily in the presence of $Ca(NO_3)_2$ in concentrations ranging from 0.001m to 0.025m, but no germination was found in combinations with 0.0005m or 0.00025m of the calcium salt. The form and abundance of germination with 0.001m $Ca(NO_3)_2$ was here much the same as that found in 0.00004m of the copper salt alone. Here a ratio of five molecules of the calcium salt to two of the copper salt, the latter occurring in the solution at a concentration of 0.0004m, reduces the toxicity of the $Cu(NO_3)_2$ so as to produce an effect on spore germination equivalent to that exercised by a 0.00004m solution of the copper salt alone. In other words, addition to a 0.0004m $Cu(NO_3)_2$ solution, of $Ca(NO_3)_2$ in the molecular ratio of five of the latter to two of the copper salt, has the same effect as diluting the $Cu(NO_3)_2$ solution to ten times its original volume.

A third series of combinations of the same two salts, but with higher concentrations, was carried out in a manner somewhat different from that employed in the preceding series. Here the concentration of $Ca(NO_3)_2$ was the same (0.05m) in all of the cultures, while that of the copper salt varied. The concentrations of $Cu(NO_3)_2$ ranged from 0.00025m to 0.01m, and the spores germinated in all the concentrations used except the highest. With a concentration of 0.008m $Cu(NO_3)_2$ germination was similar to that found with 0.00004m of the copper salt without the addition of $Ca(NO_3)_2$. From this series it appears that addition to the $Cu(NO_3)_2$ solution here used (0.008m), of about six molecules of $Ca(NO_3)_2$ for each molecule of the copper salt present in the solution, reduces the toxicity of the latter compound in the same way as though the solution had been diluted to two hundred times its original volume. It appears as though the presence of $Ca(NO_3)_2$, in this molecular proportion of about 6 to 1, altered the relations between spores and solution so that only a two-hundredth part of the copper nitrate actually present was effective to modify or retard the germination processes.

A series of combinations of $Cu(NO_3)_2$ with KNO_3 was carried out, in which a constant concentration of the potassium salt, 0.05m, was used in combination with $Cu(NO_3)_2$ in concentrations ranging from 0.0002m to 0.01m. The spores germinated in concentrations up to and including 0.002m of the copper salt. In the last mentioned concentration, the form of germination was quite similar to that found in a 0.00004m solution of the copper salt alone. Thus in a solution containing KNO_3 at a 0.05m concentration and $Cu(NO_3)_2$ at a 0.002m concentration (a molecular ratio of 25 of the potassium salt to 1 of the other), the toxicity of the copper salt is decreased to a magnitude only one fiftieth as great as is that shown by this concentration of the copper salt alone.

The results of the three series of combinations of copper nitrate with calcium nitrate which have been described, are summarized in table I, together with certain other data which require consideration.

From the data just given the question arises, whether the influence of calcium nitrate in reducing the toxicity of the copper salt may be due to a direct effect produced in the solution or to some change brought about in the spore itself. Krönig and Paul [97], in considering an effect similar to this, in which the toxicity of $HgCl_2$ upon *Bacillus anthracis* was decreased by the addition of NaCl, concluded that the change thus brought about is due to depression of the ionization of the salt of the heavy metal. Such a supposition cannot apply in the present case, however; that alteration in the ionization of $Cu(NO_3)_2$ cannot be the cause of the diminution of its toxicity as here observed, is clearly shown by simple calculation according to the isohydric principle. The percentage of dissociation of $Cu(NO_3)_2$ in the mixtures here employed should be determined by the concentration of the NO_3 radical. Following this generalization, the data given in table I (C, a) have been obtained. Seventy-six per cent. of the copper salt of the first combination is seen to be dissociated.

TABLE I

		Series I	Series II	Series III
A. $Cu(NO_3)_2$ present in the medium, *molecular*....	a. Common fraction......	$\frac{1}{125}$	$\frac{1}{2500}$	$\frac{1}{10000}$
	b. Decimal fraction......	0.008	0.0004	0.0001
B. $Ca(NO_3)_2$ required to allow same kind and amount of germination as occurs in 0.00004m (m/25000) $Cu(NO_3)_2$ without $Ca(NO_3)_2$.....	a. Common fraction, *molecular*...............	$\frac{1}{20}$	$\frac{1}{1000}$	$\frac{1}{16000}$
	b. Decimal fraction, *molecular*...............	0.05	0.001	0.0001
	c. Number of molecules of $Ca(NO_3)_2$ for each molecule of $Cu(NO_3)_2$.	6.25	2.50	0.63
C. Concentration of dissociated $Cu(NO_3)_2$ present in mixture, *molecular*................	a. According to the isohydric principle.......	0.0061	0.0004	0.0001
	b. From potentiometer measurements.......	0.0058	0.0004	0.0001
D. Concentration of dissociated $Cu(NO_3)_2$ in physiologically equivalent solution of this salt alone, *molecular*..	From conductivity measurements (Jones' tables).............	0.00004	0.00004	0.00004

Now, if only *one* per cent. of the copper here actually present in the mixture were ionized, the concentration of the Cu ion would correspond to that in an 0.00008m solution of $Cu(NO_3)_2$ alone, which, as has been shown, is stronger than is necessary to inhibit spore germination. It is therefore apparent that the decrease in the toxicity of the copper salt shown in these combinations with $Ca(NO_3)_2$ is not to be related to decreased ionization of $Cu(NO_3)_2$.

Another theory to account for a similarly decreased toxicity of $HgCl_2$

in the presence of NaCl was advanced by Clark [01], who considered that a double salt was formed, such as Na_2HgCl_4, and supposed this to be only slightly dissociated in the presence of an excess of NaCl. This writer also worked with various copper salts in the presence of KNO_3, K_2SO_4, NH_4SO_4, etc., and suggested that the decreased toxicity of the copper salts, which was uniformly observed in such combinations, was likewise due to formation of double salts.

In view of the work of Hosford and Jones,[17] on the dissociation of double salts in dilute solution, it seems very improbable that the lowering of the toxicity of $Cu(NO_3)_2$ brought about by $Ca(NO_3)_2$, as shown in the present study, is due to decreased ionization of the toxic compound, brought about by double salt formation. It is, however, possible to obtain direct evidence on this question by comparing the concentrations of copper ions in the various solutions, with and without the calcium salt. The percentage of dissociation occurring in simple solutions of $Cu(NO_3)_2$ has been determined experimentally for many concentrations, and these data are available in published tables.[18] From such data may be derived the concentration of copper ions present in any solution containing only $Cu(NO_3)_2$, but no published data are yet available from which might be ascertained the concentrations of copper ions in the binary mixtures here dealt with.

The determinations here required of the relative concentrations of copper ions in solutions of $Cu(NO_3)_2$ with and without addition of the calcium salt, may be made by means of the potentiometer.[19] With this instrument the solutions involved in table I were tested, and the differences in electrical potential between the combination solutions of $Cu(NO_3)_2$ and $Ca(NO_3)_2$ and the corresponding simple solutions of $Cu(NO_3)_2$ were measured. The two solutions to be tested (for example, the simple solution containing 0.008m $Cu(NO_3)_2$ and the combination solution containing 0.008m $Cu(NO_3)_2$ and 0.05m $Ca(NO_3)_2$) were placed, respectively, in the two arms of a U-tube, separated below by a saturated solution of ammonium nitrate. Into each arm of the U-tube was introduced a copper electrode and these were connected with a potentiometer (Leeds and Northrup, type K). The potentiometer was so calibrated that the difference in voltage between the two copper solutions might be read directly on the instrument.

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[17] Hosford, H. H., and Jones, Harry C., Conductivity, temperature coefficients of conductivity and dissociation of certain electrolytes. Amer. Chem. Jour. 46: 240-278. 1911.

[18] Jones, Harry C., Electrical conductivity, dissociation and temperature coefficients of conductivity, from zero to sixty-five degrees, of aqueous solutions of a number of salts and organic acids. Carnegie Institution of Washington Publ. 170. Washington. 1912.

[19] Loomis, N. E., and Acree, S. F., A study of the hydrogen electrode, of the calomel electrode, and of contact potential. Amer. Chem. Jour. 46: 585-620. 1911.

It is a pleasure to acknowledge here, that the suggestion of this method, as applicable to the problem in hand, is due to Professor S. F. Acree, of the Chemistry Department of the Johns Hopkins University, and that much valuable help in making the determinations was received from him and from Dr. C. N. Myers. This part of the work was carried out at the chemical laboratory.

The copper electrodes were freshly plated and calibrated, for subsequent correction of the readings, with reference to any difference in potential which might exist between them when both were placed in the simple $Cu(NO_3)_2$ solution.

The difference in concentration of copper ions in the two solutions thus compared was calculated from the observed difference in electrical potential by the following formula:

$$V = 0.0591 \ \log_{10} \frac{C}{C_1}$$

in which V is the observed Electromotive force, in volts; C and C_1 are the concentrations, respectively, of the copper ions in the two solutions, and the quantity 0.0591 is a constant (Loomis and Acree [11], page 591).

In each of the tests with which we are at present concerned three entirely separate determinations were made, for each of which the reading was several times repeated, and the results were in excellent agreement. It was found that the difference in electric potential between the 0.008m solution of $Cu(NO_3)_2$ and the same solution containing also a 0.05m concentration of $Ca(NO_3)_2$, was 0.006 volt, the simple solution having the higher potential. From this observation it appears, by substitution in the formula given above, that

$$\frac{C}{C_1} = 1.26,$$

or that the concentration of copper ions in the combination solution is $\frac{1}{1.26}$, or 0.794, as great as that in the simple one. Now, from Jones' [12] conductivity tables (page 57), it is found by interpolation that the $Cu(NO_3)_2$ in our simple solution (0.008m) is about 90.6 per cent. dissociated at 25° C. It thus appears that the $Cu(NO_3)_2$ in the combination solution here considered must be dissociated to an extent equal to 0.794×90.6, or 71.9 per cent.

It has been shown above (page 71) that the $Ca(NO_3)_2$ in this particular combination should decrease the dissociation of the copper salt, on account of the common ion, to 76 per cent. There is thus a difference of about 4 per cent., between the dissociation of the $Cu(NO_3)_2$ in this mixture as calculated from the isohydric principle and that derived from the potentiometer measurements. This difference may, of course, be due to the formation of a double salt, following the hypothesis of Clark already mentioned, but it makes no difference in the present discussion whether it be 76 or 72 per cent. of the $Cu(NO_3)_2$ that is here dissociated; as has been stated, the solution would still be toxic enough to inhibit germination in these spores if only *one* per cent. of the total $Cu(NO_3)_2$ were dissociated.

The remaining two concentrations of $Cu(NO_3)_2$ (0.0004m and 0.0001m, see table I), with and without additional $Ca(NO_3)_2$, were also subjected to potentiometer determinations of the concentrations of copper ions present therein. In neither case was there any difference in electrical potential between the two corresponding solutions. It is therefore clear that at least 99 per cent. of the $Cu(NO_3)_2$ is to be considered as dissociated in these solutions, whether the calcium salt be present or not (see table I, C, b).

From the foregoing considerations, it seems quite clear that the influence of $Ca(NO_3)_2$ in reducing the toxic effect of $Cu(NO_3)_2$ on the germination of the spores here employed is not at all to be related to any changes brought about in the solution itself by the addition of the calcium salt. It appears that this antitoxic or antagonistic influence must be effective upon the spores themselves, so altering them that they become capable of germination in solutions whose concentration of free copper ions would inhibit this process were it not for the presence of the calcium salt.

Whether the copper enters the spores and exerts its toxic action directly, through some alteration in the protoplasm, or whether this toxic influence is exerted primarily upon the spore walls or upon the surface film of the protoplasm, thus perhaps creating some disturbance in physico-chemical equilibrium that may subsequently be propagated inward, is a question for the answering of which no evidence is yet at hand. Nevertheless, the present studies have clearly demonstrated that the presence of $Cu(NO_3)_2$ alone in the medium inhibits germination if the concentration be above a very low limit, produces markedly altered forms of renewed activity if the concentration is somewhat lower, and allows normal germination only when the solution is exceedingly dilute. While these facts must be interpreted to mean that the copper salt tends to upset the protoplasmic system in some way and that it is possible for that system to be so disturbed as either to inhibit germination absolutely or to allow this process to proceed in modified form, yet much more direct evidence of such protoplasmic disturbance was frequently met with in the progress of this work. Spores in which all germinational activity had been prevented by the presence of $Cu(NO_3)_2$ frequently possessed a characteristic, coarsely granular appearance, as though a precipitate or coagulum had been formed within the protoplasm. Furthermore, this same appearance was often encountered in spores which exhibited modified germination under the influence of copper. From this it appears that the granular appearance of the protoplasm does not necessarily denote death, but to settle this point conclusively and to determine whether the production of apparent granulation might not be a post mortem effect, the following experiment was performed.

Spores which had been for eighteen hours in cultures with 0.00008m solution of $Cu(NO_3)_2$ alone were employed. In this solution no germination has ever been found, in the more than twenty separate cultures which have been observed, and the protoplasm usually appears markedly granular. The spores were transferred from the poison solution to a new drop culture with distilled water and were then returned to the thermostat. At the end of twenty-four hours, observation showed that many of the granular spores had germinated normally, although the granular appearance was still very noticeable. Further proof that the granulated appearance with which we have here to deal is not a post mortem effect of copper poisoning may be derived from the fact that the germinal tubes of spores which had germinated in a combination solution of 0.0001m $Cu(NO_3)_2$ and 0.00006m $Ca(NO_3)_2$ elongated considerably when the cultures were allowed to remain in the thermostat for a second day, although the spores showed the characteristic granulation above mentioned.

It seems probable, from these and other similar lines of evidence, that the copper of the solution penetrates the spore wall and gives rise to the precipitation or coagulation effect just described.

It may be noted here that the influence of calcium upon the toxicity of copper is of interest in connection with the problem of fungicides and fungicidal action. From the results obtained in the experiments described above, the conclusion seems warranted that only a small portion of the soluble copper in Bordeaux mixture is effective as a fungicide. Yet it is a well known fact that a long list of fungous diseases, including apple bitter-rot, can be controlled by the proper applications of this mixture to the host plants. The use of KOH instead of $Ca(OH)_2$ in preparing the fungicide should result in a much more toxic mixture, for, as brought out in the preceding experiments, potassium is only about one fourth as effective as calcium in decreasing the toxicity of copper. From the standpoint of the host, however, it is quite probable that this anti-toxic effect of the calcium is important in preventing " spray-injury " to the foliage and fruit, while the small amount of soluble copper present, is, in most cases, sufficient to prevent fungous growth.

Lead.

The nitrate of lead is considerably less toxic toward the Gloeosporium spores here employed than is that of copper. The concentration just inhibiting germination after eighteen hours was found to be about 0.004m. With a concentration of 0.002m of the lead salt, some of the spores showed slight terminal or lateral swellings. At this concentration spores were frequently found in which one half was brown or blackened while the other seemed devoid of protoplasm, appearing as if the protoplasmic contents had all collected in one end and had then taken on a thicker wall. No increase in size or local swelling was apparent in such cases. In a concentration of 0.001m the blackened bodies just mentioned were found, as well as swellings, and also definite outgrowths from one or both ends of the spore. The latter (shown in figure 3) are of particular interest and require some attention here. They are spherical in shape, dark brown or black in color and appear in all respects similar to the chlamydospores or appressoria which have been frequently described as resulting under certain conditions, from the germination of spores of various Gloeosporium forms.

Appressoria have been described by Hasselbring[20] as forming when nutrient materials are absent and when germinating spores or germ tubes come in contact with such hard surfaces as are furnished by the cover glass in a drop culture or by the epidermis of various fruits. This writer considers that they function as holdfasts and that they result from a contact stimulus acting upon spores or tubes which are not well nourished. That such bodies are frequently formed when germ tubes come in contact with the cover glass of a hanging drop culture, has often been demonstrated in the present studies. They are especially characteristic of cultures with certain salt solutions, at concentrations somewhat below that at which germination is entirely suppressed but above that at which normal development occurs. Here their formation does not appear to be related to any contact stimulus, however. In some cases swollen bodies are produced which have the form of appressoria but which are hyaline like the usual spores and germ-tubes of this fungus, thus apparently differing from the appressoria only in not being brown or black in color. In the descriptions which follow the term appressoria will be used to denote the dark colored, appressorium-like bodies, and swellings of similar form but without dark appearance will be termed hyaline appressoria. These terms are applied here merely in a descriptive way, without intended implication that the bodies thus designated may not be physiologically or otherwise different from the appressoria of the mycologists.

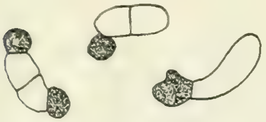

Fig. 3. Germinating spores from 0.00033m $Pb(NO_3)_2$, ×650.

In concentrations of $Pb(NO_3)_2$ from 0.0001m to 0.00005m, most of the germination observed resulted in appressoria. Some of the similar, hyaline swellings also occurred in these cultures. When cultures where appressoria were common were allowed to remain in the thermostat for several days and were examined from time to time, these swellings continued to develop successively from the same spore, until sometimes as many as four appeared together at one end (see fig. 4). In the formation of chains of these bodies it appeared, from the observation of different stages, that a second or later swelling may be brought about either by the germination of one previously formed or by enlargement of the constricted portion of the tube between one previously formed and the spore itself; in the latter case possibly by new growth from the spore. In many cases a light colored area quite like that considered by Hasselbring to be a pore, could be distinguished, and this may have been present in all cases. Spores which had germinated in drop cultures by forming appressoria were transferred to new cultures in distilled water and returned to the thermostat. Nearly all the appressoria had germinated with normal germ tubes at the end of twenty-four hours.

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[20] Hasselbring, H., The appressoria of anthracnoses. Bot. Gaz. 42: 135-142. 1906

An occasional spore germinated normally in a concentration of 0.00005m $Pb(NO_3)_2$, and a few normal tubes were found in the 0.00004m concentration, but it was only in the 0.00002m concentration that no effect of the $Pb(NO_3)_2$ upon the germination of the spores was noticeable. In the latter concentration the spores germinated with long tubes, just as in simultaneous controls in distilled water.

Series including combinations of $Pb(NO_3)_2$ with $Ca(NO_3)_2$ were carried out at three different concentrations of the lead salt, 0.00016m, 0.00033m, and 0.00066m. With a 0.00066m concentration of $Pb(NO_3)_2$ no concentration of the calcium salt was found which entirely suppressed the formation of appressoria and in which only normal germination was produced, though with a concentration of 0.05m $Ca(NO_3)_2$ only a few appressoria were found. This combination gave practically the same results as were found with a 0.00004m concentration of $Pb(NO_3)_2$ alone; i.e., somewhat over 50 per cent. of the germination was normal, though a considerable number of swellings and appressoria were formed. In the combination of the same concentration of $Pb(NO_3)_2$ (0.00066m) with 0.004m of the calcium salt the effect produced was similar to that found in cultures with 0.00005m concentration of $Pb(NO_3)_2$; only occasional normal tubes were found, these probably constituting less than 1 per cent. of the total germination. With concentrations of 0.001m, 0.002m and 0.003m $Ca(NO_3)_2$, combined with a 0.00066m concentration of the lead salt, practically no normal tubes were produced, germination resulting in appressoria and swellings. These concentrations correspond to solutions of $Pb(NO_3)_2$ alone varying in concentration from 0.001m to 0.0001m.

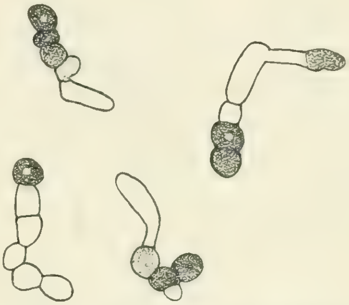

Fig. 4. Spores after four day in 0.004m $Pb(NO_3)_2$. ×650.

In the second series of combinations of $Pb(NO_3)_2$ with $Ca(NO_3)_2$, the concentration of the first salt was 0.00033m and the same concentrations of $Ca(NO_3)_2$ were employed as in the series just described. The results in these experiments were similar to those of the previous series, with some differences due to the lower concentration of the lead salt. Practically no appressoria were formed with the combination containing the 0.05m concentration of $Ca(NO_3)_2$, germination being here (see fig. 5) about the same as that which occurred with the 0.00002m concentration of the lead salt alone. With a combination containing 0.01m $Ca(NO_3)_2$, the same effect was obtained as with 0.00004m $Pb(NO_3)_2$ alone, and with combination containing 0.002m $Ca(NO_3)_2$ about the same effect was evident as with a concentration of 0.00005m $Pb(NO_3)_2$ alone. In the combinations with 0.001m and 0.0005m $Ca(NO_3)_2$ the results were practically the same as with a concentration of 0.0001m $Pb(NO_3)_2$ alone; most of the germination here gave appressoria.

With a 0.00016m concentration of $Pb(NO_3)_2$ in combination with the same concentrations of $Ca(NO_3)_2$ as were employed in the two series above described, the concentration of the calcium salt in which the majority of the germinating spores formed normal tubes (instead of swellings and appressoria), and the concentration in which only an occasional spore formed a normal germ-tube, were somewhat lower than in the previous series, as was to be expected. The combination containing a 0.002m concentration of $Ca(NO_3)_2$ gave practically the same effect as did the concentration of 0.00004m of the lead salt alone, while that containing a 0.001m concentration of $Ca(NO_3)_2$ gave results similar to those obtained with a 0.00002m concentration of the lead salt alone. Combinations containing $Ca(NO_8)_2$ in concentrations of 0.0333m, 0,04m and 0.05m, respectively, produced the same sort of germination as was obtained with a concentration of 0.00033m $Pb(NO_3)_2$ combined with a 0.05m concentration of the calcium salt; germination was practically all normal, only an occasional appressorium or swelling being found.

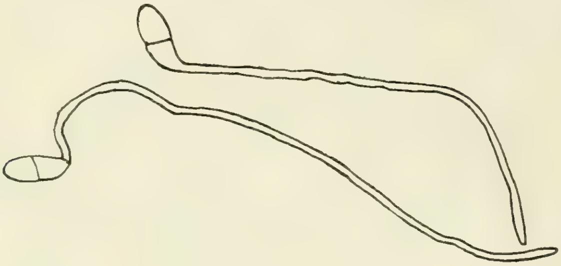

Fig. 5. Germinating spores from combination solution containing 0.00033m $Pb(NO_3)_2$ and 0.05m $Ca(NO_3)_2$ × 650.

From the above data the conclusion seems warranted that the same molecular ratio of the calcium salt to that of lead produces practically identical effects with the three different concentrations of $Pb(NO_3)_2$ here used. Concentrations of 0.01m, 0.02m and 0.06m $Ca(NO_3)_2$ combined, respectively, with concentrations of 0.00016m, 0.00033m and 0.00066m $Pb(NO_3)_2$ produce practically the same effect on spore germination as does the 0.00005m concentration of the lead salt alone. It thus appears that for all three very different concentrations of $Pb(NO_3)_2$, the addition of $Ca(NO_3)_2$ in the proportion of six molecules of the calcium salt to one molecule of $Pb(NO_3)_2$ reduces the toxicity of the latter salt to a point where it almost, but not quite, inhibits normal germination and nearly restricts germinal activity to the formation of appressoria and other swellings. To obtain this same condition of germination a simple solution of $Pb(NO_3)_2$ must have a concentration of 0.00005m.

A similar series of equivalent effects upon spore germination, with the three different concentrations of the lead salt, is shown by taking as the index of physiological activity the prevalence of normal germ tubes in the cultures, as contrasted with swellings and appressoria. This condition of affairs is attained in combination solutions containing, respectively, 0.02m $Ca(NO_3)_2$ combined with 0.00066m $Pb(NO_3)_2$, 0.01m $Ca(NO_3)_2$ combined with 0.00033m $Pb(NO_3)_2$, and 0.005m ($Ca(NO_3)_2$ combined with 0.00016m $Pb(NO_3)_2$. These three combinations of the two salts all produce practically the same effect on germination as does the concentration of 0.00004m $Pb(NO_3)_2$ alone. Here the addition of about 30 molecules of $Ca(NO_3)_2$ to 1 of $Pb(NO_3)_2$ (30:1) reduces the toxicity of the lead salt until it is physiologically equivalent to that of the 0.00004m solution of this salt alone.

Combination of $Pb(NO_3)_2$ with $Mg(NO_3)_2$ shows a similar influence of the alkaline earth upon the toxicity of the heavy metal, though with somewhat different concentrations. Equivalent physiological effects were produced by 0.00066m $Pb(NO_3)_2$ in combination with 0.02m $Mg(NO_3)_2$, by 0.00033m $Pb(NO_3)_2$ in combination with 0.01m $Mg(NO_3)_2$ and by 0.00005m $Pb(NO_3)_2$ alone. Here the addition of about 30 molecules of $Mg(NO_3)_2$ for each molecule of $Pb(NO_3)_2$ in the solution, reduced the toxicity of the latter salt so that it became equivalent, in both combinations, to a simple solution containing 0.00005m $Pb(NO_3)_2$. Under these conditions most of the germinal activity took the form of the production of appressoria and other swellings, but a small amount of normal germination was observed in all cases. Thus, $Mg(NO_3)_2$ appears to be only about one fifth as effective in reducing the toxicity of the lead salt as is $Ca(NO_3)_2$.

Magnesium nitrate was not employed in concentration sufficiently high to inhibit all toxic effects of the lead salt. The nearest approach to normal germination was obtained in a combination solution containing 0.05m $Mg(NO_3)_2$ and 0.00033m $Pb(NO_3)_2$, in which about half of the germination was normal. The main points brought out by these experiments are summarized in table II.

TABLE II

Type of germination	Physiologically equivalent solutions containing $Pb(NO_3)_2$ alone and in combination with $Ca(NO_3)_2$ or with $Mg(NO_3)_2$, *molecular*				Molecular ratio in combination Ca:Pb or Mg:Pb
	Simple solutions, $Pb(NO_3)_2$	Combination solutions containing			
		$Pb(NO_3)_2$	$Ca(NO_3)_2$	$Mg(NO_3)_2$	
Mainly normal. Some appressoria and other swellings . .	0.00004	0.00016	0.005	. . . .	30:1
		0.00033	0.01	. . . .	30:1
		0.00066	0.02	. . . .	30:1
Almost no normal germination, mainly appressoria and other swellings	0.00005	0.00016	0.01	. . . .	6:1
		0.00033	0.02	. . . .	6:1
		0.00033	. . . . .	0.01	30:1
		0.00066	0.06	. . . .	6:1
		0.00066	. . . . .	0.02	30:1

From the results obtained with the different combinations of calcium and magnesium nitrates with $Pb(NO_3)_2$, it is evident that the decrease in toxicity of $Pb(NO_3)_2$, due to the other salt, cannot be caused by a depression of the ionization of the lead salt on account of the common anion. In the combination containing 0.004m $Ca(NO_3)_2$ and 0.00066m $Pb(NO_3)_2$, about 90 per cent. of the lead salt was calculated to be in the dissociated condition. In the combination containing 0.02m $Ca(NO_3)_2$ or $Mg(NO_3)_2$ together with a 0.00066m concentration of the lead salt, the latter should be about 40 per cent. dissociated. The presence of either of these concentrations of dissociated lead salt alone in a culture solution would either prevent germination entirely or give only abnormal growth. That the decrease in toxicity may have been due to the formation of a double salt remains possible; at least no direct evidence to the contrary was obtained.

With the concentrations of $Pb(NO_3)_2$ that inhibited germination no granular appearance of the protoplasm, such as was found in otherwise physiologically similar solutions of $Cu(NO_3)_2$, was evident. Yet it is clear that the lead salt either directly or indirectly affects the protoplasm through the spore wall, as is shown by the formation of the dark bodies occupying one half of the spore in many cases. This response was observed, as has been mentioned, in the higher concentrations of the lead salt. A general discussion of the different forms of germination will be taken up below, after the effects of the combinations of calcium and magnesium nitrates with the other salts have been presented.

Zinc.

Zinc nitrate, alone and in combination with the nitrates of calcium and magnesium, was studied in much the same way as were the nitrates of copper and lead. Zinc nitrate alone inhibited germination in 0.25m concentration. Many local swellings of the spore walls were found in concentrations of 0.08m and 0.04m, while local swellings and short tubes were present in concentrations of 0.008m and 0.004m. Normal germination occurred with the concentration of 0.002m. Combinations of $Zn(NO_3)_2$ with $Ca(NO_3)_2$ and $Mg(NO_3)_2$ were tested, using a concentration of 0.04m $Zn(NO_3)_2$. In these combinations the calcium salt was employed in concentrations ranging from 0.000125m to 0.02m. Some normal germination occurred with all concentrations of $Ca(NO_3)_2$ below and including that of 0.0005m, but none was observed in the mixture containing a 0.000125m concentration of this salt. It thus appears that, normal germination of these spores may be brought about in a 0.04m $Zn(NO_3)_2$ solution, by the addition of $Ca(NO_3)_2$ in the proportion of one molecule of this to every 80 molecules of $Zn(NO_3)_2$ present.

The concentrations of $Mg(NO_3)_2$ which were employed in these combinations with the 0.04m solution of $Zn(NO_3)_2$ ranged from 0.00025m to 0.025m. Here it was found that no normal germination occurred until the magnesium salt reached a concentration in the mixture of 0.0025m. This means that to produce any normal germination in the $Zn(NO_3)_2$ solution here used, by addition thereto of $Mg(NO_3)_2$, it is necessary to add one molecule of the latter salt for every 16 molecules of $Zn(NO_3)_2$ already present. It therefore requires five times as much of the magnesium salt to counteract the toxic influence of $Zn(NO_3)_2$ in the concentration here used (0.04m) as is required of the calcium salt to produce the same effect.

The relatively small amounts of the calcium and magnesium salts, which are required to inhibit the toxic effects of $Zn(NO_3)_2$ in 0.04m concentration, preclude any possibility that the antagonistic influence of either of the former salts might be related to decreased dissociation of the zinc salt, brought about by their addition, so that this consideration needs no attention in this case.

It is interesting to recall here that the effectiveness of $Mg(NO_3)_2$ in counteracting the toxicity of $Pb(NO_3)_2$, in the two concentrations studied, so as to allow some normal germination of the spores, was also found to be only about one fifth as great as that of $Ca(NO_3)_2$. The relative effectiveness of the nitrates of calcium and magnesium thus seems to be the same whether they are employed to counteract the toxicity of $Pb(NO_3)_2$ or that of $Zn(NO_3)_2$.

Zinc nitrate did not seem to stimulate the spores to form appressoria, though hyaline swellings were common in the more concentrated solutions.

No direct evidence was obtained as to whether or not zinc nitrate enters the spores.

Aluminum.

Aluminum nitrate was used at certain concentrations, both alone and in combination with magnesium and calcium nitrates. Series were carried out, and several times repeated, including the combinations and concentrations which are presented in table III.

TABLE III

Concentration of $Al(NO_3)_3$, *molecular*		Concentration of $Mg(NO_3)_2$ or of $Ca(NO_3)_2$, *molecular*	
Common fraction	Decimal fraction	Common fraction	Decimal fraction
9/2000	0.0045	1/100	0.01
8/2000	0.004	2/100	0.02
6/2000	0.003	4/100	0.04
4/2000	0.002	6/100	0.06
3/2000	0.0015	7/100	0.07
2/2000	0.001	8/100	0.08
1/2000	0.0005	9/100	0.09
1/4000	0.00025	95/1000	0.095

In none of the cultures with $Al(NO_3)_3$ did the addition of either $Mg(NO_3)_2$ or $Ca(NO_3)_2$ bring about any alteration at all in the behavior of the spores. Although many other proportions of these pairs of salts might have been tried, it appears probable that, if either the calcium or magnesium salt, at any concentration, exerts any influence upon the toxicity of $Al(NO_3)_3$, in any concentration, some evidence in this direction would have been obtained from the experiments which were carried out. This seems still more probable from the fact that different concentrations of the aluminum salt alone produced markedly different effects upon the activities of the spores, a matter which will now receive attention.

In the highest concentration of $Al(NO_3)_3$ used in the combinations, namely 0.0045m, only a few local swellings of the spores were observed. A blackening of half of the spore was frequently found here (fig. 6). No normal germination occurred in this solution. In the two lowest concentrations of $Al(NO_3)_3$ (0.005m and 0.00025m) however, much of the germination was normal. Aluminum nitrate appears to influence the form of germination occurring in these spores, with much the same results as have been described for cultures poisoned with $Pb(NO_3)_2$. The types of renewed activity which occur with $Al(NO_3)_3$ are: (1) the formation of a dark body occupying about half of the spore, (2) the formation of a small local swelling at one end of the spore, and (3) the development of one or more appressoria, the latter sometimes having their characteristic dark color and sometimes being hyaline. Unless the formation of the internal dark bodies, which seem to arise directly through protoplasmic activity, may be taken as evidence of a penetration of the toxic salt, no information as to whether $Al(NO_3)_3$ actually enters the spores was obtained.

Nickel.

Nickel nitrate proved to be very slightly toxic to these fungus spores, its effect on germination being manifest only in very high concentrations. On this account no study was made of the possible influence of calcium and magnesium nitrates upon this toxicity.

The toxic effects of $Ni(NO_3)_2$ alone may be added here. A concentration of 0.5m inhibited germination. In a concentration of 0.25m, numerous local swellings of the spores were observed. Both local swellings and normal germination were present in a 0.125m solution of this salt, and germination was perfectly normal, as in the distilled water control, where a concentration of 0.05m or lower was employed. Germinating spores in 0.25m concentration of $Ni(NO_3)_2$ frequently exhibited a granular appearance such as has been described for spores in solutions of $Cu(NO_3)_2$ which were not quite concentrated enough to inhibit all activity.

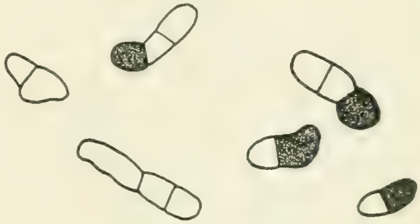

Fig. 6. Germinating spores from 0.002m Al $(NO_3)_3$, × 650.

TOXICITY OF SINGLE SALTS

The present section will be devoted to the relative degrees of toxicity exhibited by the various substances heretofore dealt with, together with certain other considerations bearing upon the problem of chemical stimulation as evidenced in the simple solutions employed in these studies. Besides the substances already mentioned, nitric acid entered into the experimentation, and the information gained regarding the toxicity of this substance will also find place here.

From what has preceded it appears possible to group the effects of the different treatments into four distinct kinds of physiological response on the part of the fungus spores here employed. (1) a given treatment may be without apparent effect: under such treatments the spores germinate normally within eighteen hours, just as they do in the control with distilled water. (2) Germinal activity may be manifest, but in other ways than that which is here considered as normal. Under treatments which produce this sort of response, the activities of the spores result sometimes (a) in an actual decrease in the exposed surface as well as in the volume of the organism, and sometimes (b) in an increase in both volume and surface, the latter, however, increasing much less markedly,—as related to increase in volume,—than is the case in normal germination. In the first category (a, above) belong the phenomena involved in the production of the dark bodies which have been described as forming within the spore wall and occupying only a portion of its volume. These appear to have essentially the characteristics of chlamydospores formed within the organism. All other renewed activities of the spores which have been described belong to the second category (b), and embrace those growth processes which result in more or less restricted swellings, especially at the ends of the spores, in appressoria, with either dark or hyaline wall, and in short germ tubes somewhat resembling the normal tubes but of much greater diameter. (3) No renewed activity may occur at all during a period of from eighteen to twenty hours under the given treatment, although ability to germinate if transferred from this treatment to another (as to distilled water) may still be retained. Here the treatment prevents germinal activity but does not destroy viability, at least within the given time limits. (4) Viability, or power to germinate in water, may be destroyed within a period of from eighteen to twenty-four hours; the organism is killed outright.

It has already become clear in this work that most of the substances dealt with produce death within the assumed time limits, if applied at a sufficiently high concentration (4). With a somewhat lower concentration of the injurious material, germination is inhibited but viability is retained throughout the given period (3). When the concentration is still lower germinal activity becomes manifest but takes other forms than those recognized as normal (2). Finally, when the concentration of the toxic agent is still further decreased, the stimulation threshold in the present sense is passed and normal germination becomes the rule (1).

Livingston[21] working with a green alga has presented a somewhat similar series of responses to chemical stimuli. This writer studied the effect of a large number of nitrates and sulphates on a form of Stigeoclonium, adding different concentrations of the salts to a nutrient medium in which the alga was grown. With a dilute nutrient solution, in which the alga had a characteristic filamentous form, its response to stimulation might be considered as of three types. (1) death, (2) change in phenomena of growth, and (3) change in phenomena of reproduction. The response to a high concentration was usually death, while addition of a toxic salt in concentration somewhat below the fatal one stimulated the production of the palmella form, with spherical cells and division occurring in all directions. It is interesting that, with relatively similar concentrations of the salts, (that is at concentrations somewhat lower than those required to inhibit germination) the spores dealt with in the present paper exhibited responses similar to those which Livingston found with his alga; the production of appressoria and swellings in this fungus appears physiologically similar to the production of the palmella form in Stigeoclonium. The third type of response discussed by Livingston, change in phenomena of reproduction, of course finds no parallel in this investigation.

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[21] Livingston, B.E., Chemical stimulation of a green alga. Bull. Torr. Bot. Club **32**: 1-34. 1905.

For convenience of comparison, the limits of the various responses of the fungus spores here dealt with, to the different treatments employed, are presented in table IV. The only nitrate occurring in the table that has not already received attention is the acid. In a series of cultures with HNO_3 the germination at a 0.005m concentration was practically all normal, while at 0.01m and all higher concentrations, no germination took place in the eighteen hour period. The acid prevented germination for the period of eighteen hours, in a 0.02m concentration and killed the spores in 0.5m. No concentration was found, therefore, at which the germination took the form of any of the various abnormal growths found at some concentration with all the other substances used, nor was there any apparent coagulation of the protoplasm at concentrations below that which killed the spores. From these considerations it seems, then, that the various abnormal growths and effects on the protoplasm of the spore cannot be due to acid present in the salt solutions, as a result of hydrolysis of the salts, but must be related, either directly or indirectly, to the metals themselves.

Turning now to table IV, the different substances are there arranged in the order of the concentration which just allowed normal germination in a period of eighteen hours. In the first column are listed the substances dealt with. The remaining columns are each double. The second column presents the concentrations in which the spores are killed in eighteen hours and fail to germinate later, after transfer to distilled water. Here, as in many other instances, the critical concentrations must not be regarded as definite in the sense of the more exact physical sciences; the concentrations employed in the experimental series were frequently rather widely separated, and, were this not the case, the variability of the organism in its resistance to toxic substances would render quite useless any attempt to define such critical points with very great accuracy. In all such work as the present, dealing with organisms, the internal conditions of the cells must be as important in determining reactions as are the external ones, and we are

TABLE IV

Substance in medium	Molecular concentration									
	Minimum in which		Maximum in which							
	spores were killed in 18 hours		spores were alive but had not germinated after 18 hours		any germination occurred in 18 hours; always abnormal		normal germination occurred in 18 hours		no toxic effect was observed	
	Common fraction	Decimal fraction	Common fraction	Decimal fraction	Common fraction	Decimal fraction	Common fraction	Decimal fraction	Common fraction	Decimal fraction
$Cu(NO_3)_2$..	1/100	0.01	1/12500	0.00008	6/100,000	0.00006	1/37,500	0.000026 +	1/50,000	0.00002
$CuSO_4$.....	1/100	0.01	1/12500	0.00008	6/100,000	0.00006	1/37,500	0.000026 +	1/50,000	0.00002
$Pb(NO_3)_2$..	......	......	1/250	0.004	1/500	0.002	1/20,000	0.00005	1/50,000	0.00002
$Al\ (NO_3)_3$..	1/5	0.2	1/50	0.02	1/200	0.005	1/2,000	0.0005	1/4,000	0.00025
HNO_3.....	1/2	0.5	1/50	0.02	......	......	1/200	0.005	1/200	0.005
$Zn(NO_3)_2$..	1/3	0.33 +	1/4	0.25	2/25	0.08	1/500	0.002	1/2000	0.0005
$Ni(NO_3)_2$..	1/2	0.5	......	......	1/4	00.25	1/8	0.125	1/20	0.02
$Mg(NO_3)_2$..	......	......	......	......	1/2	0.5	3/10	0.33 +	1/10	0.1
$Ca(NO_3)_2$..	......	......	......	......	3/5	0.6	1/2	0.5	1/10	0.1
KNO_3.....	......	......	......	......	1/1	1.0	9/10	0.9	4/5	0.8
Sucrose....	......	......	......	......	8/5	1.6	7/10	1.4	1/1	1.0

as yet unable either to control or measure the former, excepting in a very general way.

In the third column are found the concentrations at which the different salts inhibit germination for at least eighteen hours. When spores from these cultures were removed from the toxic solution and placed in water for a day, they germinated. The fourth column gives the maximum concentration in which germination occurred. In all cases this growth was abnormal, showing swellings, swollen tubes and other unusual structures, such as are illustrated in figs. 1 to 6. In the fifth column the concentrations given are the maxima in which any normal germination was observed. The sixth column presents the highest concentrations in which no toxic influence was manifest.

From table IV it will be seen that the copper salts are by far the most toxic of all the salts here studied. These two salts are very similar in their effect on the germination of the spores, which is in accord with the results of Clark [99], and points almost conclusively to the commonly accepted idea that the toxicity of such copper salts is mainly or entirely due to the copper ion. The last mentioned writer found that the concentration of copper salts which inhibited germination were higher than those producing the same effect in this investigation, a fact that may probably be due to internal differences in the organisms dealt with; he found that his five fungi varied markedly in their response to the same stimulus. Also, Clark usually made use of nutrient media, while the present studies were carried out without their employment. It is quite possible that the presence of nutrient substances might have modified the effect of the poison. Duggar[22] found the nitrate of copper considerably more toxic than the sulphate and also considerably more so than it is here shown to be. In the work of Stevens,[23] these two salts affected germination similarly and the concentrations required to prevent germination varied, with the different fungi used, from m/6400 to m/200. Many other investigations of the effect of copper on the germination of fungus spores have been carried out, with varying results. The effect of this poison on algae has been studied by Livingston [05]. Kahlenberg and True,[24], Heald,[25] Jensen,[26] Szücs [12] and others, have investigated its toxicity toward higher plants. The results obtained indicate that fungus spores are considerably more resistant toward the toxic effects of copper than are either the algae or higher plants.

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[22] Duggar, B.M., Physiological studies with reference to the germination of certain fungus spores. Bot. Gaz. **31**: 38-65. 1901.

[23] Stevens, F.L., The effect of aqueous solutions upon fungus spores. Bot. Gaz. **26**: 377-406. 1898.

[24] Kahlenberg, Louis, and True, Rodney H., On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. **22**: 81-124. 1896.

[25] Heald, F.D., The toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz. **22**: 125-153. 1896.

[26] Jensen, G.H., Toxic limits and stimulation effects of some salts and poisons on wheat. Bot. Gaz. **43**: 11-44. 1907.

In the order of their toxicity, $Pb(NO_3)_2$ follows the copper salts, though not closely, with $Al(NO_3)_3$ next. The effects of the last two salts on plants seems to have been studied but little. Livingston [05] employed them, however, and found the same relation to hold true as is here brought out. Also, Jensen [07] found that $Pb(NO_3)_2$ is somewhat less toxic toward wheat seedlings than is $Cu(NO_3)_2$. The nitrates of zinc and nickel, which are next in the order of diminishing toxicity, are not exceedingly toxic toward these Gloeosporium spores. It is interesting to note that Clark [99], working with fungi, and Livingston [05], with an alga, found nickel salts to be more strongly toxic than those of zinc, results which are opposite to those obtained in this investigation.

Magnesium, calcium, and potassium nitrates and cane sugar end the list, in the order named. It is noticeable that, with these last four compounds at concentrations just below that required to inhibit germination, growth takes the form of swollen bodies which tend to give a lower value to the ratio of surface to volume of the organism than that resulting from normal germination. This reaction to toxic stimuli, at concentrations just below those required to inhibit growth, occurs commonly with all the salts here used. That it is found with cane sugar, which is usually regarded as non-toxic (see True[27]) suggests that such reactions may be brought about by high osmotic pressure as well as by chemical stimulation in the true sense of this term. This suggestion is in accord with the conclusions of Livingston [05], who has shown that his form of Stigeoclonium assumes the palmella form in response either to a chemical stimulus or to one of relatively high osmotic pressure. Whether this effect of sugar, and similar effects produced by high concentrations of calcium, magnesium and potassium nitrates is really due, in the present instances, to osmotic pressure relations, was not determined experimentally; the minuteness of the spores must render experiments involving plasmolysis exceedingly difficult with such organisms as those here employed. It is obvious, however, that the high concentrations of the calcium, magnesium and potassium salts, found necessary to affect germination, must be capable of exerting an extremely high osmotic pressure if the ectoplasm of the spore is not readily permeable to them.

From table IV it may be seen that, with the exception of HNO_3, the different compounds arrange themselves in the same order no matter what type of reaction to the chemical stimuli may be taken as criterion. This suggests that the physiological response of death, on the one hand, and the various morphogenic responses considered in this paper, on the other, are ultimately related to the same properties or characteristics of the various stimulating substances which bring them about. Copper is the most toxic metal here dealt with, whether we consider its toxicity as bringing about changes that result in death or as bringing about the less final changes that lead to abnormal growth. In the same way, magnesium, calcium and potassium are the least toxic metals of the list, on whatever physiological criterion we may base our judgment.

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[27] True, Rodney H., The physiological effects of certain plasmolyzing agents. Bot. Gaz. **26**: 407-416. 1898.

GENERAL DISCUSSION AND CONCLUSION.

The results of the experiments described in the foregoing pages show that, in certain cases at least, the effect of a toxic salt on the germination of the conidia of *Glomerella cingulata* may be influenced by the addition, to the medium, of calcium, magnesium or potassium nitrate. That this effect is not due to depression of ionization of the toxic salt has been demonstrated. That it cannot be due to the formation of undissociated double salts has been shown for the combination of $Cu(NO_3)_2$ with $Ca(NO_3)_2$ and of $Zn(NO_3)_2$ with calcium or magnesium nitrate. The influence of calcium upon the toxicity of the salts of the heavy metals here employed must then be related to an effect of the $Ca(NO_3)_2$ on the spore, or to an effect on the contained protoplasm, as is made otherwise probable from the fact that copper, lead, aluminum and nickel nitrates sensibly effect the protoplasm in various ways, without producing any apparent changes in the spore wall.

There are, of course, a number of logically possible explanations for the antagonistic actions of salts upon the germination of fungus spores as here recorded, but any such possible hypothesis must necessarily be very complex if it be able to account generally for even the small array of facts so far at our disposal.

Various theories have been suggested from time to time to explain similar salt antagonisms. Loeb[28] has advanced the theory of ion-proteid formation, suggesting that one or both ions of a salt may combine with proteids of the protoplasm to form ion-proteid compounds. This writer found, for example, that a solution of NaCl is poisonous unless some calcium and potassium be also present in the medium, in which case the toxicity of NaCl is greatly decreased or inhibited altogether. He considers that calcium and potassium ions may to some extent take the place of the sodium ion in the ion-proteid compounds above mentioned, and suggests that the organism cannot live without some of these proteid compounds containing calcium and potassium as well as those containing sodium. The work of Osterhout[29] on balanced solutions supports this theory of Loeb.

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[28] Loeb, J., On ion-proteid compounds and their rôle in the mechanics of life phenomena. I. The poisonous character of a pure NaCl solution. Amer. Jour. Physiol. 7: 327-338. 1900.

[29] Osterhout, W. J. V. [06], On the importance of physiologically balanced solutions for plants. II. Fresh water and terrestrial plants. Bot. Gaz. 44: 259-272. 1907.

Wo. Ostwald,[30] from his studies on Gammarus, concludes that the toxicity of a solution is related to the power of the protoplasm to adsorb the dissolved substance, saying "dass eine Lösung um so giftiger ist je stärker sie adsorbiert wird." Morowitz[31] somewhat similarly considers that the toxic salt is adsorbed by the surface layer of the organism and diffuses thence into the interior, the amount thus entering in a given time being related to the rate of adsorption. He supposes that, if an otherwise indifferent but strongly adsorbed substance be simultaneously present in the medium, the adsorption of the latter decreases the power of the surface layer to adsorb the toxic salt, so that a smaller amount of this enters the cell.

True and Gies [03] also relate these antagonistic effects to protoplasmic properties, and seem to consider the antagonism between copper and calcium to be due to an accelerating effect of calcium acting against a retarding influence exerted by copper. Szücs [12], as has already been mentioned, likewise supposes the influence of one salt upon the toxicity of another to be due to simultaneous effects of the two salts upon the protoplasm.

Loeb[32] has rather recently advanced the theory that the influence of one salt on the toxic action of another is due to changes brought about in the outer colloidal membrane of the protoplasm, whereby the latter becomes impermeable to the toxic salt. Osterhout,[33] working with cut disks of Laminaria and his method of electrical conductivity, also comes to the conclusion that ([12], page 115) "the antagonistic action of salts is largely or entirely due to the fact that they hinder or prevent one another from entering the protoplasm."

It will be seen that any one of the various hypotheses just reviewed may, *with adequate quantitative assumptions*, explain the phenomena of antagonism as these are exhibited by any particular pair of concentrations of any particular pair of salts dealt with in the present study. It seems, impossible, however, to formulate a hypothesis, either by means of any of these published suggestions or with any other logical possibilities, which may simultaneously and satisfactorily account for all the cases with which we have to deal. The marked differences, brought out in the present studies, between the behavior of calcium when employed with copper and that of the same element in combination with lead, zinc or aluminum, strongly suggest the probability that valence alone does not determine the efficiency of one cation in influencing the toxicity of another. It appears that other properties must be taken into account if such differences are to be explained.

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[30] Ostwald, Wo., Ueber die Beziehungen zwischen Adsorption und Giftigkeit von Salzlösungen für Süsswassertiere (Gammarus). Archiv. Ges. Physiol. **120**: 19-30. 1907.

[31] Morowitz, Hugo. Ueber Adsorption und Kolloidfallung. Kolloidchem. Beih. **1**: 301-331. 1910.

[32] Loeb, J., The mechanistic conception of life. Chicago. 1912. Page 173.

[33] Osterhout, W.J.V., The permeability of protoplasm to ions and the theory of antagonism. Science N.S. **35**: 112-115. 1912.

Some quantitative researches on the permeability of plant cells. Plant World **16**: 129-144. 1913.

A consideration of the toxicities of the single salts, as brought out in this investigation, also suggests that the influence of each one of these salts upon the protoplasm of the spore is specific. The nitrates of lead and aluminum, in concentrations somewhat below those which inhibit germination, frequently produce within the spore (either directly or indirectly) a dark, chlamydospore-like body. The presence of copper, at a similar concentration as regards toxicity, causes a granular appearance of the protoplasm and much the same effect was observed in concentrations of nickel nitrate not quite strong enough to inhibit germination. Toxic concentrations of zinc nitrate, however, fail to produce any visible alteration in the protoplasm. The effects of the different single salts upon the spores are therefore not at all the same, and it seems at least reasonable to suppose that the requisite antidotes may not be identical and may not be effective in the same manner in all cases. A given substance at a given concentration may inhibit the poisonous effects of one toxic salt and yet have no influence upon the toxicity of another. Thus, neither calcium nor magnesium nitrate exerts any apparent influence upon the toxic effect of aluminum nitrate, but both are markedly effective in counteracting the toxicity of the corresponding salts of zinc and lead.

The conclusion seems unavoidable, therefore, that no simple and broadly general explanation can be applied to these exceedingly various antagonistic actions, and that the explanation of each particular case must involve the chemical characteristics of the salts concerned and the physico-chemical properties of their solutions. The exceeding complexity of the material system within the cell must make possible a great variety of explanations until this variety becomes further limited by increased knowledge. As has been well emphasized by Osterhout [13] the future advance of our knowledge of this intricate subject must depend solely upon further investigation of a quantitative nature.

THE LABORATORY OF PLANT PHYSIOLOGY
OF THE JOHNS HOPKINS UNIVERSITY.
APRIL 1, 1913.

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SEX AND DEVELOPMENT OF THE GAMETOPHYTE OF ONOCLEA STRUTHIOPTERIS.[1]

Elizabeth Dorothy Wuist.

ABSTRACT.[2]

This paper deals mainly with the question as to whether the nature of the sex organs of the gametophyte of *Onoclea struthiopteris* is predetermined, or whether this gametophyte may be induced by external conditions to produce either male or female organs exclusively, or both kinds of organs together. Both soil and solution cultures were employed, with various nutrient solutions in the latter, and with different intensities of insolation. From these experiments, extending over a period of several years, it appears that the sex of this gametophyte is not predetermined in the spore from which the plant developed, but that each prothallium is capable of forming both kinds of sex organs, either antheridia or archegonia, or both, according to the external conditions. It seems that both male and female tendencies are present as potentialities in the cells of the gametophyte and are influenced, in their visible expression, by the factors of the environment during the life history of the organism. The main results of the study follow.

(1) The gametophyte generation of *Onoclea struthiopteris* is shown in this study to be either monoecious or apparently dioecious, according to developmental stage and environmental conditions. In younger cultures in soil five per cent. of all the prothallia were monoecious; in older cultures, fifteen per cent. were monoecious.

(2) Ninety per cent. of the prothallia which originally bore only female organs were induced later to produce antheridia and thus become monoecious, by placing them under favorable conditions of nutrition.

(3) Five per cent. of the individuals which originally bore only male organs were induced later to produce archegonia and thus to become monoecious, by placing them under conditions of nutrition which were favorable to further growth and development.

(4) Young archegonia were formed after fertilization had taken place.

(5) Contrary to the observations of Prantl, the position of the prothallia in the soil cultures did not influence the kind of sex organs developed.

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(6) The male tendency appeared to be latent in all parts of the apparently female gametophyte, as shown by the later development of antheridia from various cells and regions.

(7) Both apparently male and apparently female prothallia were obtained in cultures of Prantl's solution from which nitrogen had been omitted.

(8) Rubidium hydrate, apparently by hindering growth, increased monoecism.

(9) Good growth and development of these gametophytes occurred in solutions without either sodium or calcium.

(10) Adventitious outgrowths were developed by the prothallia, in response to unfavorable environmental conditions.

(11) Rhizoids and reproductive organs were frequently borne upon the dorsal surfaces of the prothallia.

INTRODUCTION.

The following investigation was undertaken with the view of determining whether the gametophyte of *Onoclea struthiopteris* is always dioecious and, if it is not, what may be the environmental conditions necessary for the development of dioecious and monoecious prothallia. Later, this was extended to an experimental study of the gametophyte generation. Here the physiological phase, rather than the morphological, was emphasized, the latter being considered only so far as it had direct bearing upon the former.

The experimental work of this paper was done during the past four years, in the Botanical Laboratory of the University of Michigan. My thanks are due Dr. C. H. Kauffman, who suggested the problem and under whose guidance and inspiration the work has been carried on. I am also indebted to Professor F. C. Newcombe for many valuable suggestions and criticisms.

HISTORICAL.

The development of the gametophyte generation of ferns has, until recently, been studied more extensively from the morphological than from the physiological point of view. A historical review of the earliest literature relating to the morphology is given by Hofmeister.[3] In more recent years the literature has become very extensive; but, as it is concerned with the morphology of the prothallia of a number of genera, mention will be made here only of such work as has a direct bearing upon this study. Campbell[4] is the only one who has worked out in detail the morphology of the gametophyte and sporophyte generations of *Onoclea struthiopteris*.

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[3] Hofmeister, W., Higher Cryptogamia. Translated by Frederick Currey. London. 1862.

[4] Campbell, D.H., The development of the ostrich fern. Mem. Boston Soc. Nat. Hist. 4, No. 2. 1887.

Brief studies of the conditions controlling the sex of the gametophyte generation of this fern have been published by Mottier[5] and myself;[6] while Terletzki [84] and Milde [53] confined their study to the sporophyte generation.

The physiological study of the development of the gametophyte began with a study of the germination of the spores. The literature relating to this phase of study has been so recently summarized by Heald [98], Shultz [02], Life [07], Laage [07], Burgerstein [08] and Fischer [12], that reference need only be made to these summaries.

The first work upon the physiological development of these organisms was a study of the bilaterality of the prothallia, and the effect of light upon the formation of the reproductive organs. Leitgeb[7] demonstrated, by cultivating fern gametophytes on a nutritive solution, that the bilaterality of the prothallia was due to the effect of light, not gravity, as had been thought by some of the earlier writers. He found by altering the illumination that it was possible to produce changes in both sides of the prothallia. He also found that the reproductive organs were always borne on the shaded side. Two years later, Prantl,[8] working with *Aneimia phyllitidis, Polypodium vulgare, Osmunda regalis* and *Aspidium filix-mas*, verified the results of Leitgeb in regard to the bilaterality of the prothallia and the position of the sexual organs. In addition, he reported that strong light tended to produce only archegonia and weak light only antheridia. Klebs,[9] with strong light, obtained large heart-shaped prothallia bearing both kinds of organs. Under the action of weak light the peripheral cells of his prothallia grew out into filaments, the ends of which in some cases developed prothallia when placed in strong light. He also showed that, by reducing the light intensity, vegetative growth could be prolonged and the reproductive organs, both male and female, repressed. Heim[10] carried this line of investigation still further, experimenting with strong and weak white light, and with the yellow, blue and violet rays of the spectrum. His results with *Aneimia phyllitidis* were as follows: Strong light produced heart-shaped prothallia bearing archegonia and antheridia. Less intense light produced elongated prothallia, also bearing archegonia and antheridia. In yellow light long narrow prothallia developed, bearing antheridia but no archegonia. The blue and violet rays checked growth and produced only adventitious prothallia. Life,[11] with cultures of *Dicksonia aprifolia, Ancimia phyllitidis* and some other ferns, verified in the main the results of former work. His results indicated that light intensity affected the form of the prothallia and the formation of reproductive organs. Strong light favored the development of heart-shaped gametophytes in all the ferns studied by him except *Ancimia,* and it also favored the production of both antheridia and archegonia except in *Alsophila pruinata,* where only archegonia were produced. Weak light produced "filamentous or ribbon-like prothallia." It inhibited the development of archegonia and favored the production of antheridia. Life's experiments also indicated that young stages of *Alsophila pruinata, Ancimia phyllitidis* and *Gymnogramme* are strongly and positively heliotropic. Peirce[12] found that the prothallia of *Gymnogramme* grown from spores in Knop's solution, on a klinostat, were dorsiventral. He believes that the direction of illumination and not mechanical resistance determines the direction of growth of the germ tube and of the first cell divisions. He also believes that light determines which side of the prothallus shall develop the rhizoids and reproductive organs. Peirce's experiments showed that when the illumination was approximately equal and sufficient on all sides, the rhizoids and sexual organs developed on both sides of the prothallia. Furthermore, insufficient light suppressed the development of reproductive organs in this fern.

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[5] Mottier, David, Note on the sex of the gametophyte of Onoclea struthiopteris. Bot. Gaz. **50**, 209. 1910.

[6] Wuist, Elizabeth D., The physiological conditions for the development of monoecious prothallia in Onoclea struthiopteris. Bot. Gaz. **49**: 216. 1910.

[7] Leitgeb, H., Ueber Bilateralität der Prothallien. Flora **60**: 174. 1877.

[8] Prantl, K., Ueber den Einfluss des Lichtes auf die Bilateralität der Farnprothallien. Bot. Zeitg. **37**: 713, 719. 1879.

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[10] Heim, Carl, Untersuchungen über Farnprothallien. Flora **82**: 328. 1896.

The effect of nutritive conditions upon the development of prothallia and reproductive organs was first investigated by Prantl.[13] He concluded that the prothallia in crowded regions of soil cultures were "ameristic" and produced only antheridia, while in less crowded regions "meristic" prothallia developed and bore only archegonia. From spore cultures of *Polypodium vulgare, Aspidium filix-mas, Ceratopteris thalictroides* and *Osmunda regalis,* on nutrient solution, he reports that the prothallia in cultures without nitrogen remained "ameristic" and bore only antheridia, while those in cultures of the full solution were "meristic" and bore archegonia. From these observations Prantl drew the general conclusions that the development of the prothallia, depending on the food supply, and the separation of the reproductive organs, go hand in hand; that "ameristic" prothallia develop antheridia while "meristic" prothallia develop archegonia; that lack of nitrogen hinders the development of a meristem, and that, therefore, an insufficient nitrogen supply may account for the fact that thickly grouped prothallia bear only antheridia.

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[11] Life, A.C., Effect of light upon the germination of spores and gametophytes of ferns. Missouri Bot. Garden Report **18**: 109. 1907.

[12] Peirce, G.J., Studies of irritability in plants. Ann. Bot. **20**: 449. 1906.

[13] Prantl, K., Beobachtung über die Ernährung der Farnprothallien und die Verteilung der Sexualorgane. Bot. Zeitg. **39**: 753. 1881.

EXPERIMENTAL

A. TECHNIQUE.

Few details of technique which were applicable to my work could be gleaned from earlier sources; therefore the cultural methods used in this experimental work have been the gradual outgrowth of my own experience. It is only by experience that the value of careful and painstaking attention to details of technique can be understood and appreciated.

1. Materials.

The spores used in the early part of the work were collected from two sources: from sporophytes growing on lawns about Ann Arbor, Michigan, and from sporophytes growing under natural environmental conditions in woods about two and a half miles from that city. A careful comparative study, under various conditions, was made of cultures with spores from the two sources, to see whether any differences might be noticed, either in the germination of the spores, or in the development of the prothallia and their reproductive organs. No differences were observed, however, and only spores collected from the lawns were used throughout the remainder of the work.

Collections of fertile fronds were made about the first of October of each year. They were allowed to dry by exposing them before an open window, for from a week to ten days, depending upon the condition of the fronds when collected and that of the weather. For future use the fronds were wrapped in oiled paper and preserved in a covered pasteboard box in a cool dry place. The spores were prepared for sowing by shaking a frond over paper or a clean glass plate and the sporangia obtained were crushed with a scalpel or a microscope slide, care being used not to crush the spores. The crushed mass was then rubbed through a sieve which separated the spores from the remains of the sporangia. A sieve for this purpose was constructed by stretching four thicknesses of silk bolting-cloth (obtained from a flour mill) in a small embroidery hoop. By adding or removing a thickness of cloth, the grade of the sieve could be readily adjusted.

2. Soil Cultures.

The leaf mould used in the soil cultures was taken from a wood in which no ferns grew. New flat pots or pans, 5½ inches in diameter and 2 inches deep, were sterilized dry for from ten to fifteen minutes, at a temperature of 200° C. The pots were then filled to within an inch of their tops with leaf mould of medium fineness. This was carefully packed and smoothed, and a half inch layer of very fine soil was then spread over and pressed down to give a firm, smooth surface. Here great care was needed; if the soil layer was too fine or too firmly packed, there was a tendency, in the subsequent alternate wetting and drying, for the surface to harden and crack. After the contents had been saturated with distilled water, the pots were covered with glass plates and sterilized with steam for about ten minutes, at a temperature of 120° C. It was found that the prothallia did not thrive as well upon leaf mould which had been sterilized for a longer time. As soon as the pots were cooled, the contents were again saturated with sterilized distilled water, and the spores were sown.

Of the various methods tried for sowing the spores, the most successful was the following. A mass of spores was taken on the point of a broad-bladed scalpel, and the instrument was moved over the pot, about half an inch above the soil surface, while the spores were gently blown upon. In this manner the spores were fairly evenly distributed.

The cultures were covered with sterilized glass plates and placed in the greenhouse, where they were exposed to direct sunlight. The first watering was done from below, thus preventing the spores from being washed into masses. Sterilized distilled water was placed in glazed saucers in which the pots stood, but an excess of water was never allowed to stand in the saucers. After germination began, watering was accomplished from above by allowing water to flow slowly down the inner surface of the pot wall. The amount of moisture present was always carefully regulated and the cultures were never watered unless the appearance of the soil indicated that this was necessary. During germination and the early development of the prothallia, it was necessary to water the cultures every day; later, when the prothallia were more fully developed, it was found best to allow the cultures to become more nearly dry at times. The moisture conditions were also regulated by raising and lowering the glass covers. On dark, cloudy days, when the temperature of the greenhouse was relatively high and the atmosphere moist, the covers were raised about one-half inch by inserting pieces of wood between them and the rims of the pots. On bright days, with lower temperature and humidity, the covers were lowered. Some workers have successfully regulated the supply of moisture to such cultures by sinking the pots in boxes of wet sand or sphagnum moss, but in all the cultures of prothallia for my own experimental work, as well as for class use, the best results have been obtained by keeping the pots themselves as dry as possible. This could best be done by keeping the contents of the pots from being too moist, and leaving the pots exposed to the air and sunlight. Very little trouble was experienced with algae or fungi. If such organisms appeared on the outer or inner surfaces of the pots the latter were cleaned at once by swabbing the surface with cotton saturated with alcohol. If fungi appeared in the cultures themselves, the infested part was immediately removed with a sterilized, sharp-pointed scalpel or forceps. Certain chemical solutions have been used by other workers to free such cultures from algae and fungi. Miss Twiss,[14] working with cultures of *Aneimia* and *Lygodium*, used potassium permanganate solution to prevent growth of algae. She found a solution containing 0.015 g. of potassium permanganate in 3.5 liters of water very successful for this purpose. It did not seem best, however, in this experimental work, to introduce any such substance, which might possibly affect the final results.

3. Solution Cultures.

In preparing the solution cultures, 26 cc. of a nutrient solution, to which had been added a drop of one per cent. solution of ferric chloride, was poured into a small, sterilized glass capsule. The spores were sown upon the solution, and the cultures thus prepared were placed in the greenhouse, under the same light and temperature conditions as prevailed for the soil cultures. Once a week the prothallia were transferred to fresh solutions, this being done by simply lifting them, with a sterilized seeker, from the old to the new solution. Care had to be exercised not to immerse the prothallia in the solution, for if this accidentally occurred it required considerable skill to make them remain on the surface afterwards; however, with older prothallia this could sometimes be accomplished by quickly drying the upper surfaces with filter paper.

A liter of each nutrient solution was made up, from Kahlbaum's pure chemicals, and these solutions were kept in flasks. In the beginning of the work each solution was carefully sterilized before it was used; it was decided later, from experimental evidence, that the cultures did better on unsterilized solutions—probably because of chemical changes caused by heating. This was especially true of solutions containing ammonium nitrate. As the stock flasks were kept well stoppered with cotton and in a clean place, and as care was used in opening them, little trouble was experienced with contamination by growths of algae or fungi.

The formulas used in making up the nutrient solutions here employed are given below.

I. Beijerinck's Solution.

NH_4NO_3	0.5 g.
KH_2PO_4	0.2 g.
$MgSO_4$	0.2 g.
$CaCl_2$	0.1 g.
$FeCl_3$	trace.
Distilled water	1000 cc.

II. Knop's Solution.

$MgSO_4$	0.25 g.
$Ca(NO_3)_2$	1.00 g.
K_2HPO_4	0.25 g.
KCl	0.12 g.
$FeCl_3$	trace.
Distilled water	1000 cc.

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[14] Twiss, Edith M., The prothallia of Aneimia and Lygodium. Bot. Gaz. 49: 168. 1910.

III. Sachs' Solution.

KNO_3	1 g.
NaCl	0.5 g.
$CaSO_4$	0.5 g.
$MgSO_4$	0.5 g.
$CaHPO_4$	0.5 g.
Distilled water	1000 cc.

IV. Prantl's Solution. (Prantl [81].)

K_2SO_4	0.7 g.
NaCl	0.23 g.
$CaSO_4$	0.7 g.
$MgSO_4$	0.5 g.
Na_3PO_4	0.5 g.
NH_4NO_3 solution, 0.064 per cent	20 cc.

V. Rubidium Solution. (original)

RbOH	0.2 g.
$Ca(NO_3)_2$	0.5 g.
KH_2PO_4	0.5 g.
$MgSO_4$	0.5 g.
$FeCl_3$	trace.
Distilled water	1000 cc.

The following modified series of the above solutions were also used; items not mentioned are the same as in the corresponding unmodified solution.

Knop's Solution.

II a RbOH, 0.2 g. added.
II b $MgSO_4$, 2.5 g.; KH_2PO_4, 2.5 g.; KCl, 1.2 g.
II c KH_2PO_4, 0.37 g.
II d $Ca(NO_3)_2$, 0.50 g.; $MgSO_4$, 0.74 g.; KH_2PO_4, 2.00 g.
II e $Ca(NO_3)_2$, 0.50 g.; $MgSO_4$, 2.00 g.; KH_2PO_4, 0.74 g.
II f $Ca(NO_3)_2$, 0.74 g.; $MgSO_4$, 0.50 g.; KH_2PO_4, 2.00 g.
II g $MgSO_4$, 1.00 g.; KH_2PO_4, 1.00 g.
II h $Ca(NO_3)_2$, 1.00 g.; $MgSO_4$, 1.00 g.; KH_2PO_4, 1.00 g.

Sachs' Solution.

III a KNO_3, 5.00 g.

Prantl's Solution.

IV a NH_4NO_3 omitted.
IV b NaCl and Na_3PO_4 omitted.
IV c NaCl omitted.
IV d Na_3PO_4 omitted.
IV e NaCl, 2.3 g; NH_4NO_3, 6.4 g.
IV f NH_4NO_3 omitted; NaCl, 2.3 g.
IV g NaCl and Na_3PO_4 omitted; NH_4NO_3, 6.4 g.
IV h NaCl omitted; NH_4NO_3, 6.4 g.
IV i Na_3PO_4 omitted; NaCl, 2.3 g.; NH_4NO_3, 6.4 g.

Rubidium Solution.

V a $Ca(NO_3)_2$ omitted.
V b RbOH, 0.5 g.; $Ca(NO_3)_2$, 0.8 g.; KH_2PO4, 0.8 g.
V c RbOH, 0.6 g.
V d RbOH, 0.6 g.; KH_2PO_4, 3. g.
V e RbOH, 1.00 g.; $Ca(NO_3)_2$, 1.2 g.; KH_2PO_4, 1.2 g.; $MgSO_4$, 1.2 g.
V f RbOH, 2.5 g.; $Ca(NO_3)_2$, 1.1 g.; KH_2PO_4, 2.5 g.; $MgSO_4$, 2.5 g.

Cultures were also made by sowing the spores upon sterilized distilled water in small glass capsules and exposing them to direct sunlight in the greenhouse. Ten days later, when the prothallia had attained a growth of ten cells, one hundred were transferred, with sterilized seeker and dissecting microscope, to each of the following nutrient solutions, as above described, and modifications of these as shown below.

I. Beijernick's Solution, unmodified.
I a NH_4NO_3 omitted.
I b KH_2PO_4 omitted.
I c $CaCl_2$ omitted.

II. Knop's Solution, unmodified.
II a RbOH, 0.2 g. added.

III. Sachs' Solution, unmodified.
III a KNO_3, 0.5 g.

IV. Prantl's Solution, unmodified.
IV a NH_4NO_3 omitted.
IV c NaCl omitted.

V. Rubidium Solution, unmodified.

VI. Birner and Lucanus' Solution, modified.[15]

$MgSO_4$	0.5 g.
$Ca(NO_3)_2$	1.5 g.
KH_2PO_4	1.0 g.
Fe Cl_3	trace.
Distilled water	1000 cc.

These cultures were placed under the same conditions of light and temperature, and were given the same care and attention as the older solution cultures. By thus germinating the spores upon distilled water, and transferring the young prothallia to nutrient solutions, the number placed upon each solution was definitely known. At the same time, even the smallest fragments of sporangia were eliminated from the cultures. A question might be raised in regard to a possible shock, which prothallia might receive when transferred from distilled water to nutrient solutions, but the results obtained with these transfer cultures were practically the same as when the spores were sown directly upon the nutrient solutions, and it does not seem necessary to lay stress upon this point.

B. CULTURE STUDIES.

The germination of spores and the development of prothallia and reproductive organs will now be considered. The study of the germination of the spores, in both soil and solution cultures, was emphasized only as a means of determining the most favorable physiological conditions for rapid germination.

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[15] Birner and Lucanus' [66] solution contains, unmodified, 1.1 g. of ferric phosphate, but a trace of the chloride is here substituted for the phosphate. With repeated trials the chloride proved much more satisfactory.

1. Soil Cultures.

Germination began in the soil cultures from four to six days after the spores were sown. Growth was rapid but not uniform for the different prothallia, some developing more rapidly than others from the very beginning. Reproductive organs appeared about six to eight weeks after the spores were sown. Monoecious, as well as dioecious[16] prothallia were observed. At this time, about one to five per cent. of all the prothallia were monoecious, the others seemed regularly dioecious. The monoecious prothallia were not as large as the females but were of medium size, heart-shaped, notched, and bore archegonia on a well developed meristem, and antheridia on the surface and margins of the wings. The cellular structure was apparently good and they appeared to be normal, healthy gametophytes. (Figures 1 and 2.)

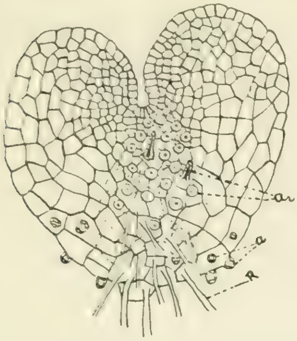

Fig. 1. Surface view of monoecious gametophyte from soil culture. *a*, antheridia; *ar*, archegonia; *R*, rhizoids.

Among the apparently dioecious prothallia, the females were large, distinctly heart-shaped and notched, with a meristem and numerous archegonia in various stages of development; the younger archegonia being near the apex, the older ones near the base of the prothallium (Figure 3). The male prothallia varied in size and shape; some were small and irregular, with but one layer of cells, and bore antheridia on both surfaces and along the margins. These did not appear to be in a healthy condition; few chloroplasts were present in the cells. Other male prothallia were regular in shape, somewhat elongated, with a slightly heart-shaped apex; these also bore antheridia on both surfaces and along the margins (Figures 4, 5 and 6).

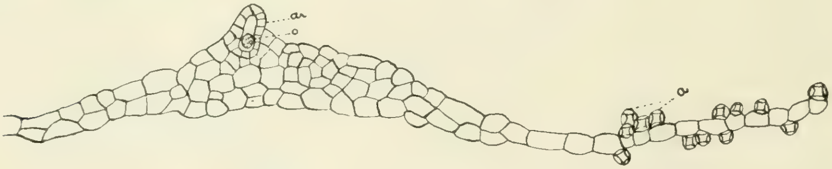

Fig. 2. Transverse section of monoecious gametophyte similar to that of Fig. 1. *a* and *ar* as in Fig. 1; *o*, oospore.

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[16] In order to avoid possible ambiguity it may be remarked that the terms *male* and *female* are employed throughout this paper in no other than a purely descriptive sense. Gametophytes which are apparently dioecious, such as have formerly been thought to produce only male or female organs, will thus be called male or female. These prothallia with but one kind of sexual organs will be termed *dioecious*, also in a purely descriptive sense. Such forms become obviously monoecious if, later, they form organs of the opposite sex.

The position of the prothallia in the cultures did not seem greatly to influence either their development or their sex, contrary to what Prantl [81] found for certain other ferns. Large, heart-shaped, female prothallia were frequently found in crowded regions, while male prothallia occurred in less crowded regions and monoecious prothallia were found throughout the cultures. Occasionally slight elevations were accidentally formed on the surface of the leaf mould, and upon some of these only male prothallia were found, with female prothallia crowded about the base of the elevated area. Upon others, the reverse was true, the archegonial prothallia being closely grouped on the elevation, with males scattered at the base. Likewise, in some slight depressions formed at the sides of the pots, female prothallia could be found grouped together, in others only the male form.

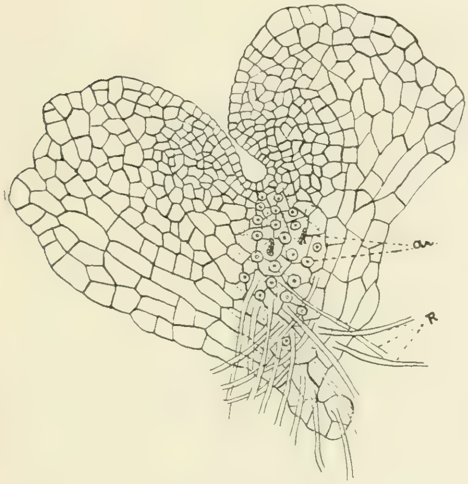

Fig. 3. Surface view of dioecious, female gametophyte from soil culture. *ar*, archegonia; *R*, rhizoids.

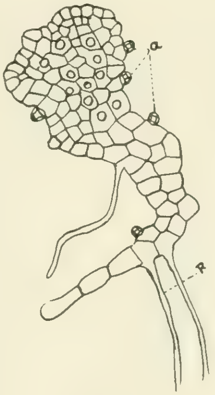

Fig. 4. Surface view of dioecious, male gametophyte from soil culture. *a*, antheridia; *R*, rhizoids.

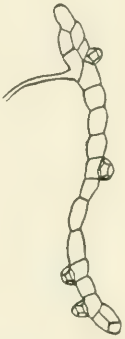

Fig. 5. Very small male gametophyte from soil culture.

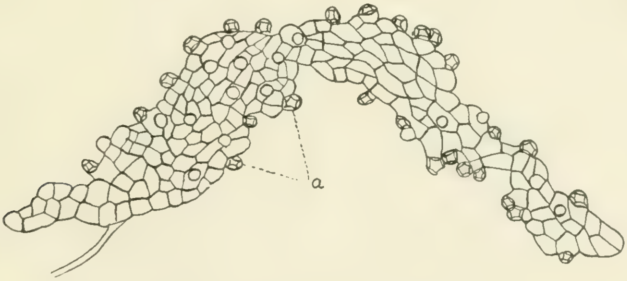

Fig. 6. Large male gametophyte from soil culture. *a*, antheridia.

Twelve to sixteen weeks after the spores were sown, the percentage of monoecious prothallia in the cultures was greatly increased. Sometimes as much as fifteen to twenty per cent. of the individuals were monoecious.

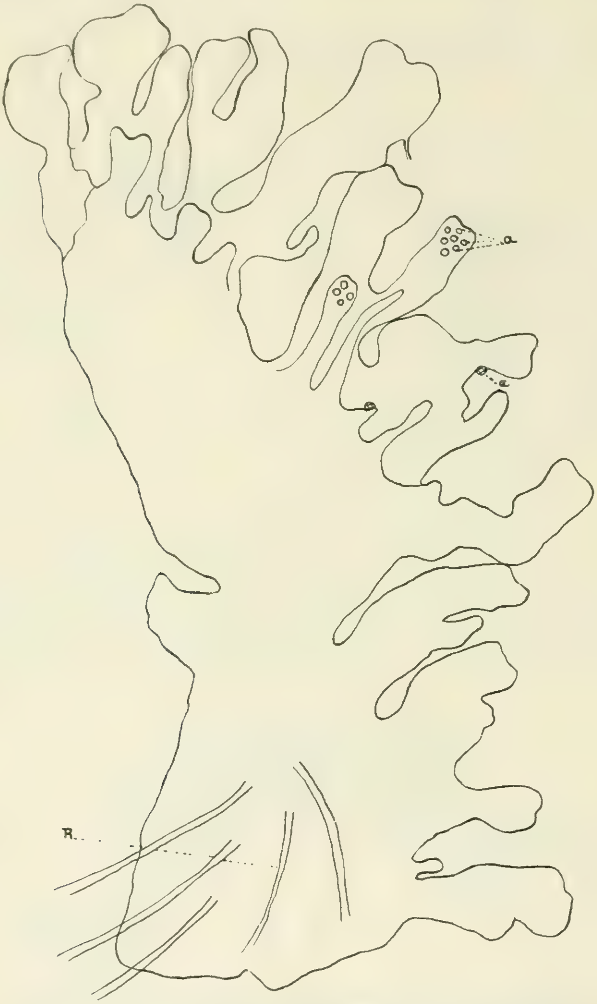

Fig. 7. Outline of gametophyte neglected for a time and then rejuvenated, showing irregular outgrowths bearing antheridia. *a* antheridia; *R*, rhizoids.

This percentage nearly approaches that of the latest count (12 per cent.) given by Mottier [10]. While the majority of these monoecious prothallia were in good condition, some were not as healthy; the latter appeared to be merely older female prothallia which had become monoecious by the development of antheridia upon their surfaces and along the margins.

Some of these same cultures, from which many of the prothallia had been removed for experimental purposes, were later placed on an interior laboratory table and neglected for about two weeks. At the end of that time all the prothallia appeared dead. The pots were then placed under bell-jars near the window and kept well watered. In about a week outgrowths began to form from the margins of the old prothallia, and six to eight weeks afterward these outgrowths had developed into new prothallia of various shapes, which were sometimes monoecious and sometimes dioecious. Some of these dioecious outgrowths were filamentous in form and were covered with antheridia. Other outgrowths developed into elongated prothallia bearing archegonia, which, in turn, became monoecious, either by developing antheridia on the surface and margin or by developing adventitious shoots bearing antheridia. (Figure 7.)

2 Solution Cultures.

The time of germination, both in nutrient solutions and in sterilized distilled water, was approximately the same as that in the soil cultures. Four days after the spores were sown germination began. Slight differences in the early development of the prothallia could be noted in these cultures. In distilled water, in most instances, there was a tendency for three cells to be formed by transverse divisions; these cells not infrequently became markedly elongated before the terminal one was divided by an oblique longitudinal wall. Thus the prothallia assumed a more or less filamentous form. In the nutrient solutions of lower concentration, as a rule, only two cells were formed before longitudinal division began, the second cell being soon divided by an oblique longitudinal wall. No noticeable elongation took place in either of these two cells. In solutions of higher concentration, the division of the cells in the young gametophytes followed that occurring in solutions of less concentration, but the shape of the cells differed from those formed either in solutions of lower concentration or in distilled water by being very short and broad. Thus, the young prothallia immediately assumed a flattened form, no filamentous or protonemal stage being observable.

The following results are based upon the study of the prothallia as they developed in the nutrient solution cultures previously described. As has been remarked, no fundamental differences were noted at any time, between the prothallia developed from spores sown directly upon nutrient solutions and those transferred at early stages from sterilized distilled water to nutrient solutions. The period over which most of these observations extended, varied from twelve to sixteen weeks for each set of cultures. (Figures 8, 9, 10.)

Solution I (*Beijerinck's solution, unmodified*). In the younger cultures all of the prothallia were dioecious, while in the older cultures about one-third of the female prothallia became monoecious by the development of proliferations bearing antheridia. The average female was large, ranging in size from 6 x 3 mm. to 7 x 3 mm. The male prothallia were of the usual size and shapes. Well developed sporophytes were present in all the other cultures.

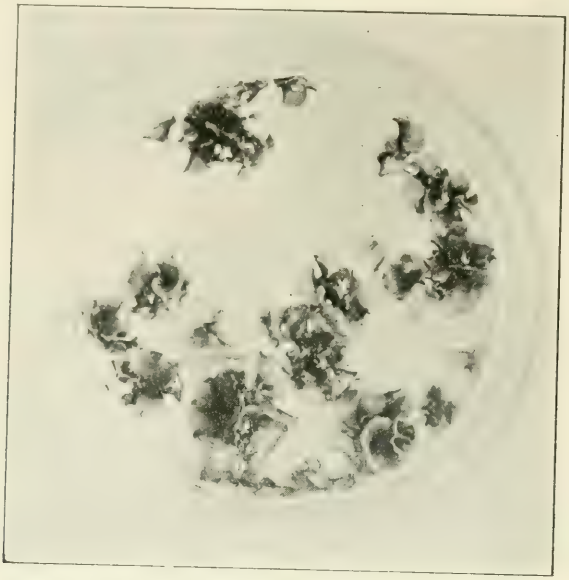

Fig. 8. Surface view of a nutrient solution culture, showing the prothallia.[17]

Solution Ia. No monoecious prothallia were observed. All the prothallia were small and, as in the cultures of solution IVa, about one-third of the individuals were females and two-thirds males. The female prothallia resembled those in solution IVa, in size, shape and general appearance, but were more distinctly notched and generally bore fewer archegonia.

Solution Ib. The prothallia were small, poorly developed and short-lived. Antheridia, but no archegonia were formed.

Solution Ic. All the prothallia were dioecious. The proportion of males to females, the size, shape, general appearance and development of the reproductive organs were the same as in solution I.

Solution II (*Knop's solution, unmodified*). Germination of the spores, and growth of prothallia were very good. In the young cultures more than two-thirds of the prothallia were dioecious. The majority of these were females, heart-shaped, notched, and with well-developed meristems and numerous archegonia in various stages of development. The average size was 6.5 x 3 mm. However, a large number of monoecious individuals were observed in the older cultures. These resembled the females in size, shape and general appearance. Most of the males were regular in shape, elongated, with slightly heart-shaped apex. Vigorous young sporophytes were present. One of these sporophytes was kept alive five months, and developed five fronds, one of which was 2.5 cm. in length. (Figure 11.)

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[17] This and the other photographic prints here employed were made by Miss Frances Dunbar.

In some of the cultures in solution II the prothallia were never transferred to fresh solutions, but were allowed to develop upon the same solutions on which the spores had been sown. Here the average size was approximately the same as when the transfer had occurred, but the percentage of monoecious individuals was much larger. Of the dioecious prothallia, about two-thirds were males and one-third females.

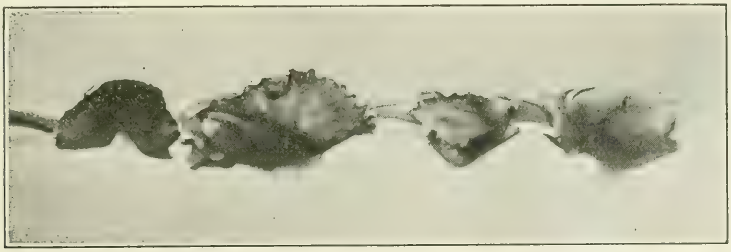

Fig. 9. Side view of portion of the same culture as is shown in Fig. 8.

Solution IIa. Germination of spores and early growth of prothallia were rapid; later growth was slower. Twelve weeks after the spores were sown *sixty per cent. of the prothallia were monoecious.* Among the dioecious individuals, there were more males than females. Both the monoecious and the female prothallia were heart-shaped and distinctly notched, and varied in size from 5 x 2 mm. to 4 x 2 mm. The monoecious individuals were normal and in a healthy condition. The female prothallia appeared healthy, but bore fewer archegonia than the average female of other cultures. The male prothallia were of the usual sizes and shapes. No sporophytes were observed.

Solution IIb. Both in growth and development the gametophytes closely resembled those in solution II. The average size was somewhat larger than in that solution, but the proportion of monoecious to dioecious prothallia in young and old cultures was the same. In cultures where the nutrient solution had not been changed, the prothallia were smaller than in the corresponding culture with solution II, the average size was only 5 x 2.5 mm. and the percentage of monoecious individuals was the same. Sporophytes were present in all the cultures.

Solution IIc. The spores failed to germinate in either the cultures in full light or those in reduced light.

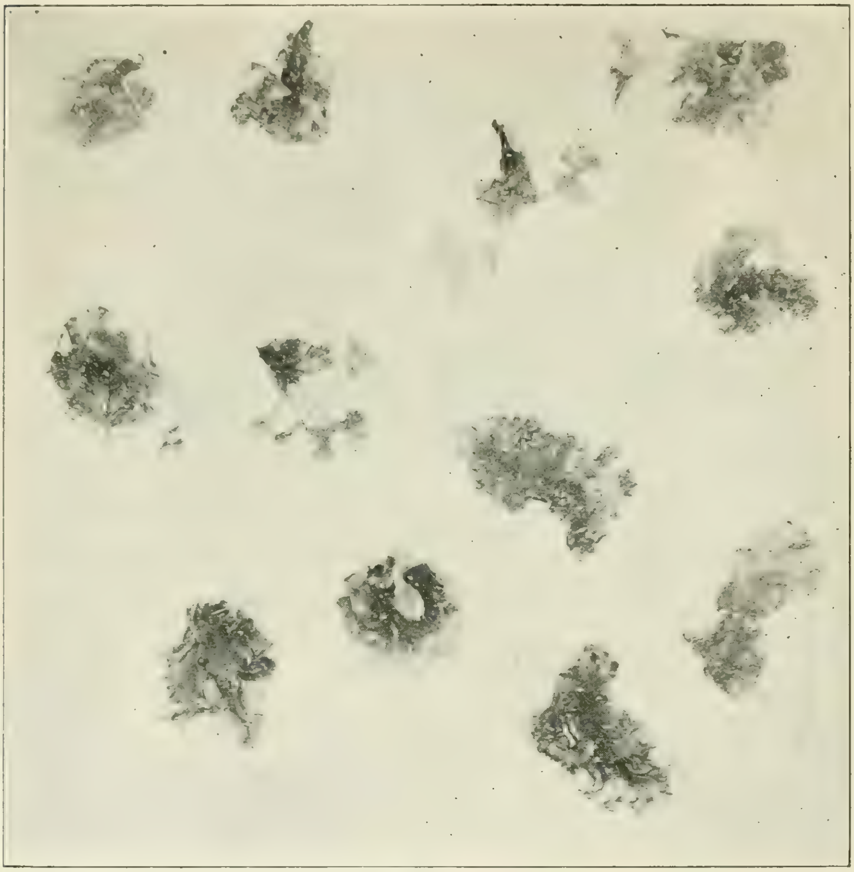

Fig. 10. Prothallia and young sporophytes from various nutrient solution cultures.

Solution IId. No germination occurred in any of the cultures.

Solution IIe. No germination in any of the cultures.

Solution IIf. No germination.

Solution IIg. No germination.

Solution III (Sachs' solution, unmodified). The prothallia were regularly dioecious and most of them were females. They were heart-shaped, notched, and averaged about 8 x 3 mm. in size. All seemed normal and in excellent condition. The male prothallia, which were few in number, resembled those of other cultures. Young sporophytes in all stages of development were present.

Solution IIIa. Growth and development were rapid and no monoecious prothallia were observed. More than two-thirds were female, and were very distinctly heart-shaped, notched and generally larger than the female prothallia in solution III. The prothallia appeared healthy, the cells were very turgid, and many young sporophytes were developed.

Solution IV (Prantl's solution, unmodified). Germination of spores and growth of prothallia were excellent. The first antheridia and archegonia were observed six weeks after the spores were sown. Seven weeks later more than two-thirds of the prothallia were females and ranged in size from 10 x 5 mm. to 5 x 4 mm. They were distinctly notched, with a well developed meristem bearing archegonia in various stages of development. Archegonia were not infrequently found upon the dorsal surface. *In these cultures, as in the soil cultures, new archegonia were formed long after fertilization had taken place.* The cellular structure of the prothallia and the development of the rhizoids were normal, though rhizoids were sometimes found upon the dorsal as well as upon the ventral surface. A few monoecious individuals occurred in older cultures, these being of the same size and general appearance as the females. All the male gametophytes were small here, and varied in shape, some being irregular and some elongated with slightly heart-shaped apices. Young sporophytes in various stages of development were observed; some of these lived three or four months upon the solutions and formed a well-developed root and several fronds.

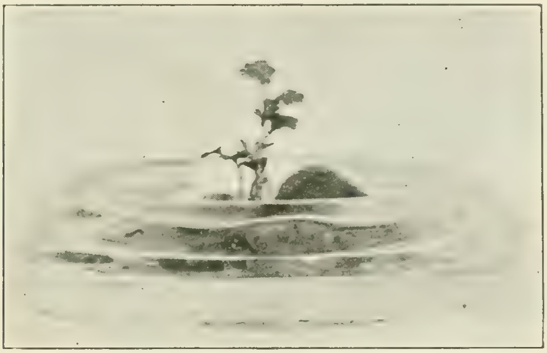

Fig. 11. Young sporophyte growing in Knop's solution.

Solution IVa. The time required for spore germination and the rate of growth of the young gametophytes for the first two weeks, were here approximately the same as in the complete Prantl's solution (IV). From this time on growth was slower; by the end of the third week these prothallia were only one-half as large as those in the full solution. Three weeks later, at the same time as in the full solution cultures, antheridia and archegonia were observed. About one-third of the prothallia were females and two-thirds males. Greater uniformity in size and shape was here apparent, among both males and females, than in any of the other solution cultures. The average size of the prothallia was about 2 x 1.5 mm. The majority were heart-shaped, very slightly notched and appeared to be in perfectly healthy condition. Rhizoids were here much more numerous and much longer than in any other of the solution cultures. The female prothallia had a well-developed meristem on which the archegonia were borne. Occasionally an individual was found with one or two archegonia on the dorsal surface. No monoecious prothallia were observed. That the reproductive organs were perfectly normal, was shown by the presence of many young sporophytes. (Figures 12 and 13.)

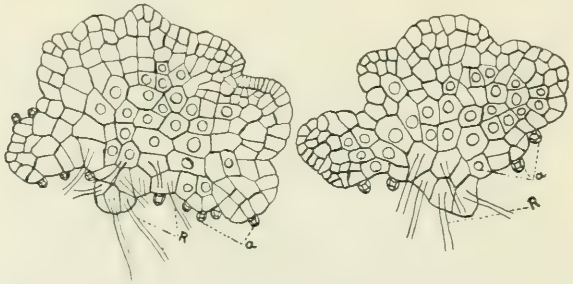

Fig. 12. Dioecious, male gametophytes from solution IVa, without nitrogen. *a*, antheridia; *R*, rhizoids.

Solution IVb. Both germination of spores and growth and development of prothallia and reproductive organs were perfectly normal. The cellular structure appeared good. The majority of the prothallia were female, of the characteristic shape, and varied in size from 7 x 4 mm. to 4 x 1.5 mm. Male prothallia of the two types, regular and irregular, were present in larger numbers than in the complete Prantl's solution (IV). A few monoecious individuals were present. Sporophytes in various stages of development were also present.

Solution IVc. Germination and development were the same as in the full Prantl's solution (IV). Although the majority of individuals were dioecious, more monoecious ones were observed in these cultures than in those of the full solution. The female prothallia ranged in size from 7 x 3 mm. to 3 x 1.5 mm. The monoecious prothallia resembled the females in size and shape, but some of them exhibited structural peculiarities. Antheridia frequently developed on the meristem, among the archegonia, and one case was observed in which they developed from the neck cells of archegonia. Several archegonia were observed with single egg cells and two distinct necks. The male prothallia showed no unusual form or structure.

Solution IVd. Spore germination was very good. The growth of prothallia was not rapid and the cultures, as a whole, did not appear to be healthy. The reproductive organs appeared at the usual time, the majority of the prothallia being females and varying in size from 6 x 3 mm. to 4 x 2 mm. The male prothallia were of the two forms above mentioned. A few monoecious individuals were observed. Young sporophytes were also present.

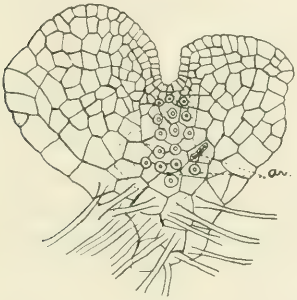

Fig. 13. Dioecious, female gametophyte from solution culture as in Fig. 12. *ar*, archegonia.

Solution IVe. Most of the plants were dioecious, a few monoecious ones were observed. The monoecious and female individuals varied in size from 8 x 3 mm. to 5 x 2 mm. and all were in healthy condition. Both types of male prothallia were present. Numerous young sporophytes developed.

Solution IVf. Spore germination and later growth and development were approximately the same as in solution IVa. The proportion of male to female individuals was also the same as that in solution IVa, but the uniformity in size and shape among the prothallia was not here as great. The females were a trifle larger and more distinctly notched than those in solution IVa, the average size being 3 x 1.5 mm. The males varied in size and shape and their structure seemed normal, although they appeared distinctly yellowish. Fewer and shorter rhizoids were developed than in solution IVa No monoecious prothallia were here observed. Young sporophytes were present.

Solution IVg. The majority of these prothallia were female and averaged in size from 2 x 1.5 mm. to 3 x 1.5 mm., the largest measuring only 5 x 2.5 mm. The males were of the usual size and shapes. A few monoecious individuals were observed. All of the prothallia appeared normal and in healthy condition. Many young sporophytes were present.

Solution IVh. These prothallia showed the best growth and development found in the series. Almost all were female, the largest being 10 x 3 mm., while the average size was 8 x 3 mm. Most of the male prothallia were of the regular type. No monoecious ones were observed. Many young sporophytes were present.

Solution IVi. Growth was fair, with a larger percentage of females than males. The average size of the females was from 4 x 2.5 mm. to 5 x 2.5 mm. No monoecious prothallia were observed. Young sporophytes were present.

For each of the following rubidium solutions besides the cultures kept in the bright light, an additional series was subjected to slightly reduced light. The apparatus used for obtaining different degrees of shading consisted of two ground glass boxes made by joining together the ends of four pieces of ground glass with gummed cloth strips ("passe partout"), subsequently well coated with asphalt varnish. This glass framework stood upon a ground glass plate and was covered with another similar plate. When it was desired to reduce the light still more these glass chambers were covered with white cheese-cloth fastened at the edges by gummed cloth strips.

Solution V (Rubidium solution, unmodified). One week after the spores were sown both sets of cultures showed slight indications of germination. Seven days later, germination had advanced somewhat in the full light cultures, the young prothallia being usually one to two cells in length. In the reduced light cultures there were no further indications of germination. At the end of two weeks two-thirds of the germinating spores in the full light were dead, while the remainder, which had reached a two or three celled stage, were unhealthy and soon died. All spores in the reduced light cultures were dead at this time.

Solution Va. Seven days after sowing a splitting of the outer spore case indicated the commencement of germination. In the cultures in reduced light the indications were more pronounced than in the others. One week later both series of cultures showed single celled prothallia each with a well developed rhizoid. By the end of the third week the individuals of all cultures had five or six cells, but they never developed further and soon died.

Solution Vb. Most of the spores in both series of cultures showed a splitting of the outer wall, but germination did not go further in the cultures in reduced light. In full light a few spores germinated, but these died in about three weeks, after the formation of two or three cells.

Solution Vc. Neither in full light nor in reduced light did germination ever advance beyond a splitting of the outer spore case.

Solution Vd. No germination occurred.

Solution Ve. Fourteen days after the spores were sown one-third of them, in both light intensities, had developed one cell with a rhizoid. Those in the full light never developed further, while those in reduced light lived a week longer, producing two or three cells before death ensued.

Solution Vf. In the full light cultures germination had just begun one week after the spores were sown, while in the cultures in reduced light there were then already indications of the formation of rhizoids. One week later, only about one-third of the germinating spores in full light had begun to form rhizoids. In the reduced light the majority of the young prothallia had by that time produced from one to three cells. At the end of three weeks, all of the germinating spores in full light were dead, while those in reduced light had developed six or seven cells, but they died soon after.

3. Induced Monoecism.

Monoecious prothallia were produced by transferring male and female prothallia from the soil cultures to nutrient solutions, and from one solution to another. The prothallia were selected from the soil cultures under a reading glass supported by a clamp on a ring stand, a dissecting microscope proving unsatisfactory because of the limited field of vision and the difficulty of focusing upon the soil cultures. The reading glass gave a larger field and furnished a good working distance between it and the surface of the culture. The prothallia were removed from the soil with a sharp-pointed scalpel and placed in a drop of water on a slide. The soil particles were first removed under a dissecting microscope, and the objects were then carefully examined with low power of a compound microscope. As the prothallia would not float upon the solutions, it was necessary to supply some supporting medium. For this purpose cotton was first used but its liability to decay in the solutions rendered it unsatisfactory; also, the growing rhizoids became entangled among the cotton fibers and were apt to be injured when the prothallia were removed for examination or transfer to fresh solutions. Glass wool was tried and likewise proved unsatisfactory; aside from unpleasantness in handling, the rhizoids were injured as they grew about in the wool. Glass beads of two sizes were used very successfully as a support for the larger female prothallia, but could not be used for supporting the smaller males. Small beads, 2 mm. in diameter, proved best. An advantage in the use of such beads was the ease with which they could be cleaned and sterilized, a disadvantage was the difficulty in packing them close enough together so that they could not roll slightly when the cultures were moved, thus allowing the prothallia to slip down between them. Sand proved the most satisfactory supporting medium in many ways, especially for the small male gametophytes. Perfectly clean, pure, white sand was placed in small glass capsules and saturated with the nutrient solution, none of the solution being allowed to stand above the sand surface.

Transfer of female gametophytes from soil to solution.

The female prothallia transferred from the soil to the nutrient solutions were approximately normal and healthy. They were large, heart-shaped, distinctly notched, with well developed meristem bearing archegonia in different stages of development.

The following nutrient solutions, prepared according to the formulas already given (see page 99), were here employed: I (Beijerinck's solution), Ia, Ic; II (Knop's solution), IIc IId, IIe IIf, IIg; Vb (Rubidium solution, modified) and Ve. With each of these solutions three series of cultures were prepared, series 1 kept in bright light, series 2 in slightly reduced light (in the ground glass box) and series 3 in reduced light (in the glass box covered with white cloth). The results from these cultures were as follows, after from four to twenty-eight days.

Solution I (Beijerinck's solution, unmodified). Series 1. The majority of the prothallia becam e monoecious in from four to seven days after the transfer. Antheridia were produced both from marginal cells and from ventral surface cells on the wings, near the apex. Death immediately followed in the first prothallia transferred. This seemed to be caused, in some cases by too long exposure to bright sunlight, in others by injury brought about in transferring. Later, by improved technique, the latter difficulty was overcome.

Series 2 and 3. The majority of the individuals became monoecious after from fourteen to twenty-eight days, antheridia being produced in large numbers, both from the marginal cells and those of the ventral surface. Some prothallia gave rise to numerous proliferations, either in the form of filaments bearing antheridia at their apices, or in thallus form, the latter being distinctly notched and bearing antheridia from the marginal and ventral surface cells.

Solution Ia. No results.

Solution Ic. Series 1. No results.

Series 2 and 3. Practically the same results were obtained here as in series 2 and 3 with the complete solution (I).

Solution II (Knop's solution, unmodified). Series 1 and 2. No results were obtained. About one-half of the individuals transferred in each series lived and appeared to be in a normal healthy condition.

Series 3. Seventy-six per cent. of the prothallia transferred lived and fourteen per cent. became monoecious. All but one were in a healthy condition.

Solution IIc. Series 1. Sixty per cent. of the prothallia transferred lived and forty per cent. became monoecious. These were in a fair condition.

Series 2. Fifty-five per cent. of the prothallia transferred lived and nine per cent. became monoecious. These prothallia appeared healthier than those in series 1. New archegonia were formed.

Series 3. No results. Approximately one-half of the individuals died.

Solution IId. No results. All the prothallia lived and the majority appeared in good condition.

Solution IIe. Series 1. All the prothallia lived, ten per cent. became monoecious. The monoecious prothallia did not appear as healthy as the female prothallia.

Series 2 and 3. No results. All the prothallia lived and were in a healthy condition.

Solution IIf. Series 1 and 2. No results. All the prothallia lived and were in good condition.

Series 3. All the prothallia lived and twenty per cent. became monoecious. These were in poor condition.

Solution V (Rubidium solution, modified). Series 1 and 2. No results.

Series 3. Ninety-eight per cent .of the prothallia transferred lived and thirty-one per cent. became monoecious. All were unhealthy.

Solution Ve. Series 1. Fifty-five per cent. of the prothallia transferred lived and nine per cent. became monoecious. The monoecious prothallia did not appear to be as healthy as the females.

Series 2. Fifty-one per cent. of the prothallia transferred lived and six per cent. became monoecious. None of the prothallia appeared to be healthy.

Series 3. Sixty-nine per cent. of the prothallia transferred lived and eleven per cent. became monoecious. All were in poor condition.

Effect of chloroform. With the aim of determining whether chloroform might induce monoecism, four sets of cultures, each of ten female prothallia, were made with solutions I and Ve, and placed in an atmosphere containing chloroform. These cultures, each with a control without chloroform, were exposed to the following light conditions: bright light, slightly reduced light (in the glass box), reduced light (in the glass box covered with white cloth) and darkness. Three days later only two monoecious prothallia were found in the cultures in the atmosphere of chloroform and two in those of the controls. From this it appears that chloroform is not effective to produce monoecism, at least in the concentration and with the other conditions here employed.

Transfer of male gametophytes from soil to solution.

The male prothallia transferred from the soil to the nutrient solutions were of the two kinds previously described; one being irregularly shaped, with one layer of cells and bearing antheridia on both surfaces and on the margins, while the other was more regular, somewhat elongated, with a slightly heart-shaped apex, also bearing antheridia as above. The second type appeared much more vigorous and healthy than the irregularly shaped ones. All the individuals were approximately ten to twelve weeks of age when transferred. The same methods of selection and transfer were employed as for the transferred female prothallia; the only supporting medium that proved satisfactory was sand. Over one hundred prothallia were transferred to Beijerinck's and Knop's solutions; one series of cultures was placed in bright light, another series in slightly reduced light, and a third series in reduced light. All of the small, irregularly shaped prothallia died. Four of the regularly shaped individuals lived, and these became monoecious in four to six weeks after they had been transferred. Three of these grew somewhat in length, and the apices broadened and developed into distinctly heart-shaped, notched prothallia with meristems bearing archegonia on the ventral surfaces. (Figure 14.) The fourth prothallium, which was originally more heart-shaped and less elongated than the other three, became monoecious by the development of a meristem upon which were borne the archegonia. (Figure 15.)

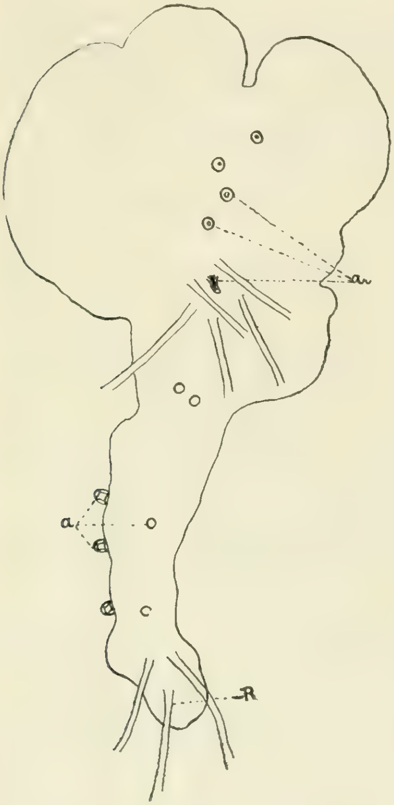

Fig. 14. Male gametophyte transferred from soil culture to solution and supported by pure sand, showing new growth (enlarged end) bearing archegonia. *a*, antheridia; *ar*, archegonia; *R*, rhizoids.

Transfers from one solution to another.

A further attempt to produce monoecism was made by transferring female and male gametophytes from one nutrient solution to another, the cultures standing in bright light. The characterization of the transfers and the resulting responses are presented below.

Transfer of females.

1a. From solution IV to solution I. No results. Growth greatly stimulated.

1b. From solution IV to solution Ic. No results. Growth as above.

1c. From solution IVa to solution I. No results. Growth as above.

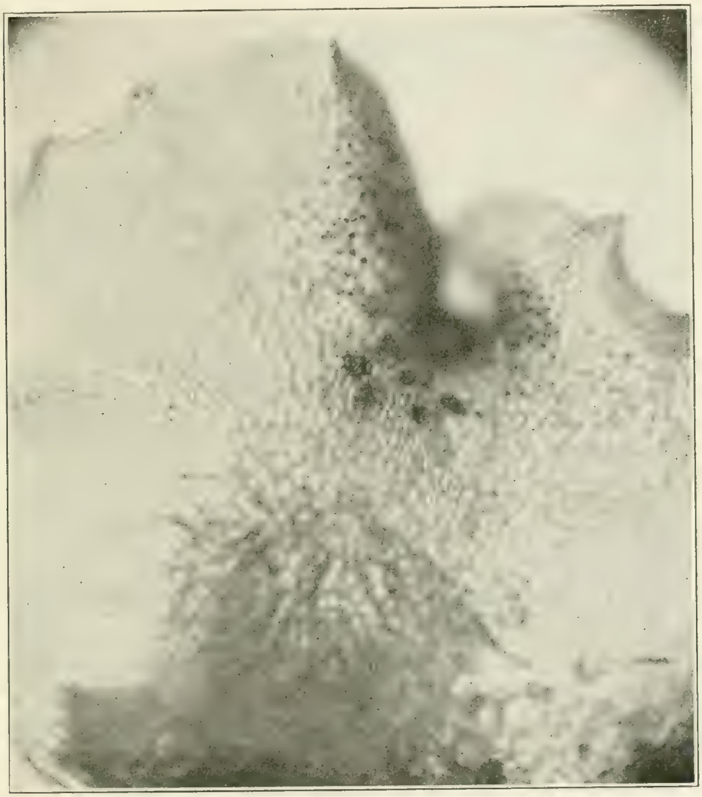

Fig. 15. Portion of male gametophyte transferred from soil to Knop's solution and supported by pure sand, showing advent of monoecism by production of archegonia, also showing antheridia.

2a. From solution II to solution Ib. Monoecious prothallia were obtained.

3a. From solution VI to solution Ia. No results.

3b. From solution VI to solution II. No results.

4a. From solution III to solution II. Monoecious prothallia were obtained; adventitious shoots and young sporophytes were developed.

4b. From solution III to solution I. No results.

4c. From solution III to solution Ic. No results.

5a. From solution IIIa to solution I. No results.
5b. From solution III to solution Ic. No results.

Transfer of males.

The male prothallia transferred from one nutrient solution to another, were regular in shape, either elongated or heart-shaped, slightly notched, one layer of cells in thickness, and bore antheridia on the surfaces and margins. The following transfers were made.

1. From solution IVc to solution IV.
2. From solution IVg to solution IVc.
3. From solution IVi to solution IVe.

Four weeks after the transfer the prothallia in all the cultures became monoecious, by the development of a meristem bearing archegonia. All had grown, and varied in size from 4 x 3 mm. to 5 x 3 mm. While distinctly heart-shaped, most of them were only slightly notched.

GENERAL DISCUSSION.

The discussion of the different phases of this investigation may be presented under three headings: (A) Vegetative growth and development, (B) Sexual reproduction and (C) Sex latency.

A. VEGETATIVE GROWTH AND DEVELOPMENT.

As previously stated, the study of the germination of the spores was emphasized only for the purpose of determining the most favorable physiological conditions for rapid germination. The time of germination was found to be the same in the soil and in the nutrient solution cultures, with the exception of solutions Ve and Vf, of the rubidium series. Here, the higher concentrations of rubidium hydrate seemed to exert the same inhibitory influence upon germination as was exerted by the lower concentrations upon the later growth and development of the prothallia. The best germination was obtained in all the cultures by exposing them to bright sunlight.

The rate of growth was approximately the same in both soil and solution cultures during the early stages of development. Later, growth varied somewhat, especially in the nutrient solutions. Mature prothallia in the solutions favorable to growth were, on an average, larger than those in the soil cultures.

Most of the nutrient solutions used seemed to favor good growth, but detailed examination of the results given above shows certain prominent differences in form, growth, and development between the different cultures. This is in keeping with the results of other investigators, for fern prothallia as well as for many other lower plants. Klebs' [93] work clearly demonstrated this. Osterhout,[18] in his experimental work on balanced solutions, found various differences in the form and growth of thallus and rhizoids in the liverworts and in Equisetum, depending on the nutrient medium. Livingston[19] observed two very distinct and easily distinguishable forms of Stigeoclonium according to the culture medium in which the alga was grown.

Several important facts were brought out by the growth and development of the gametophytes in solutions IVa and IVf (of the Prantl series). In solution IVf ammonium nitrate was omitted and the amount of sodium chloride was increased from 0.23g. to 2.3g., in the formula given on page 100.

The first of these facts demonstrates the extremely small amount of nitrogen which is necessary for the full development of prothallia and reproductive organs in this plant. Nitrogen is known to be one of the elements required by plants, and, since it has not yet been demonstrated that fern prothallia have the power of utilizing free nitrogen, it appears that the very small amount of this element present in the spore and spore case must have been sufficient, in these experiments, for the needs of the prothallium throughout its life history. Another fact shown by these cultures was a great increase in the number and length of rhizoids. A further discussion of this point will be taken up in a later paragraph. A third point of importance was the accelerating influence exerted upon growth by an increased concentration of sodium chloride in the absence of ammonium nitrate. This is especially interesting as Osterhout [09] has demonstrated that an antagonism exists between sodium and ammonium.

Sodium, as was to be expected, did not seem to be necessary for either growth or the development of reproductive organs. In fact, the very best growth was obtained in solutions in which this element was not included. When present it gave more favorable results as phosphate than as chloride. The early experiments of Birner and Lucanus[20] showed that sodium sulphate favored the entrance of phosphoric acid into the plant; while it lowered the rate at which calcium was taken up. By the addition of sodium chloride to the nutrient solution, calcium, sulphur and phosphorus were absorbed more slowly, but the percentage of magnesium and potassium in the plant was increased. Benecke[21] suggests that sodium takes the place of potassium in osmosis, thus helping to sustain turgor in the plant. Copeland[22] also ascribes to sodium the power of acting as either a direct or an indirect factor in sustaining turgor. He observed poor growth but high turgor when phosphorus, magnesium, and sulphur were omitted; while in the absence of potassium, growth was stunted and turgor decreased. Hartwell, Wheeler and Pember's experiments[23] with seedlings of millet, barley and rye indicated that the addition of sodium gave no increase in growth when potassium was abundant, but when potassium was deficient in the culture the addition of sodium proved beneficial. Schimper[24] considered the salts of potassium and oxalic acid, in the absence of calcium, to be poisonous. Based upon this consideration, Hartwell, Wheeler and Pember [07] suggest that sodium may possibly unite with oxalic acid as the latter is carried from one part of the plant to another, the acid being subsequently precipitated as the calcium salt. Osterhout [09], in a series of experiments on algae, liverworts, Equisetum and some thirteen genera of the flowering plants, demonstrated that both sodium and potassium could exert toxic effects; while Livingston [00] observed that sodium was a little more poisonous than potassium for certain green algae. Kauffman[25] in his work on the *Saprolegniaceae,* found that sodium and ammonium phosphate exerted, as they did in Klebs' experiments,[26] an inhibitory action upon the formation of reproductive organs in *Saprolegnia mixta;* no antheridia or oogonia were formed. Kauffman also observed that, when sodium chloride was added to a haemoglobin solution along with potassium phosphate, it exerted a stimulating effect upon reproduction, which was shown by a decided improvement in the appearance of the oogonia and by an increase in the number of antheridia.

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[18] Osterhout, W.J.V., The nature of balanced solutions. Bot. Gaz. 47: 148. 1909.

[19] Livingston, B. E., On the nature of the stimulus which causes the change of form in polymorphic green algae. Bot. Gaz. 30: 289. 1900.

[20] Birner, H., and Lucanus, B., Wasserkulturversuche mit Hafer in der Agric.-chem. Versuchstation zu Regenwalde. Landw. Versuchsst. 8: 140. 1866. (Quoted from Wheeler and Hartwell [06].)

[21] Benecke, W., Untersuchungen über den Bedarf der Bakterien an Mineralstoffen. Bot. Zeitg. 65: 1. 1907.

[22] Copeland, E.B., The relation of nutrient salts to turgor. Bot. Gaz. 24: 399. 1897.

The nitrates, either of potassium, ammonium, or calcium, when increased in concentration up to a certain limit, tended to stimulate growth in these fern gametophytes; above this limit, however, these salts exerted an inhibitory effect. This was especially true of ammonium nitrate. When the concentration of ammonium nitrate in solution IV (Prantl's) was increased from 0.64g. to 6.4g. per 1000 cc. of solution, the prothallia never reached maturity; growth was very slow, and the form was greatly distorted. The best growth was obtained with potassium or calcium nitrate. In solutions where the amount of potassium nitrate was increased, growth was very rapid and more nearly uniform for all individuals, and turgor was especially pronounced. The effect produced by the omission of nitrate from the solution has already been discussed. It was also observed that if a nitrate was supplied, ammonium or calcium could be omitted without materially affecting either the rate or amount of growth. This seems to indicate, either that ammonium and calcium were not necessary for the development of the prothallia, or that the small amount of these substances present in the spore and spore case proved sufficient for the needs of the organism. The results obtained when calcium was omitted are especially interesting, as Reed[27] found that spores of *Gymnogramme sulphurea* germinated and produced normal prothallia in solutions without calcium salts. By substituting magnesium for the missing calcium in his solutions, Reed obtained good growth; antheridia were produced but no archegonia. Other experiments by the same writer indicated further, that a small amount of calcium sufficed for the nutrition of most plants and that this element was beneficial in its power as an antidote for magnesium. Schimper [90] and Groom [96] attributed the function of calcium to the neutralizing of oxalic acid, while Kraus [97], Loew [02], Bruch [02], and Grafe and von Portheim [06] opposed this view. Benecke [07] found, in experiments with Spirogyra, that an antagonism existed between magnesium and calcium. Loew [99] and Kearney and Cameron [02] agreed with this view. Molisch [96] found calcium necessary for wall formation in Spirogyra. Kauffman's cultures of Saprolegnia in calcium nitrate and calcium phosphate solutions showed several cross walls separating the hypogynous cell from the oogonial stalk. The recent work of Osterhout has shown, without a doubt, that an antagonism exists not only between sodium and calcium, but also between magnesium and calcium.

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[23] Hartwell, B.L., Wheeler, H.J., and Pember, F.R., The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. Rhode Island Agric. Exp. Sta. Report **20**, part 2: 299. 1907.

[24] Schimper, A.F.W., Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora 73: 207. 1890.

[25] Kauffman, C.H., A contribution to the physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs. Ann. Bot. **21**: 361. 1908.

[26] Klebs, G., Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896.

Another point that is worthy of consideration is the effect of rubidium hydroxide as shown in the present studies. This substance was used in connection with Knop's solution (IIa) and also in a special series of solutions in which the amount of rubidium hydrate, as well as of the other compounds, was varied (Va. Vf). Germination failed in the latter series, so conclusions must be based upon the results obtained with the former. No difference could be noted between cultures in unmodified Knop's solution and those with addition of rubidium hydrate, either in the germination of the spores or the early growth of the gametophytes. Later, growth became slower, however, and the fully developed prothallia were smaller in the presence of the rubidium compound. Monoecism was greatly increased by rubidium hydrate, as shown by the fact that *sixty per cent. of the prothallia* in solution IIa were monoecious. It appears that the rubidium compound here exerted a retarding effect upon growth and that the vitality of the prothallia was thus sufficiently lowered to stimulate a latent male tendency to activity and yet not kill the organisms. There has been much discussion regarding the effect of rubidium salts upon plants and the ability of these salts to replace potassium in plant nutrition. Birner and Lucanus [66], with water cultures of oat plants, found potassium to be absolutely indispensable for growth and development, and that it could not be replaced by rubidium, caesium, sodium, lithium or ammonium. These results were confirmed by the observations of Hellriegel [07]. Benecke [07] found rubidium capable of replacing potassium in the nutrition of *Bacillus fluorescens* and *B. pyocyaneus.* Molisch's [96] work showed that for *Protococcus infusionum* the chemically related elements rubidium, caesium, lithium and sodium could not be substituted for potassium. Loew,[28] working with lower plants, demonstrated that rubidium could replace potassium. He also found that when all necessary elements, including potassium, were present rubidium exerted an accelerating effect upon growth. Kauffman's [08] cultures of Saprolegnia in haemoglobin solutions to which rubidium hydroxide had been added, showed good vegetative growth but the development of sexual organs was here inhibited. All this evidence seems to indicate that the effect of rubidium is not the same in all cases, but varies with the nature of the plant.

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[27] Reed, H., Value of certain nutritive elements to plant cells. Ann. Bot. **21**: 501. 1907.

Since the early work of Leitgeb [77] and of Prantl[29], which demonstrated the dorsiventrality of fern prothallia, the lower surface, as the shaded side, has been considered the normal region for the development of rhizoids and reproductive organs. The majority of the rhizoids in my experiments, however, developed from the upper surfaces of the prothallia although these surfaces were fully exposed to bright light. This was especially true in the solution cultures, where water was always present on the lids of the capsules. It was also true in the soil cultures which were kept so moist that drops of water collected on the glass plates covering the pots. Light conditions were suggested by Peirce [06] as controlling the origin of rhizoids upon the various sides of prothallia of *Gymnogramme triangularis,* but such a supposition will not prove satisfactory here; the gametophytes of my experiments did not receive equal illumination on the two sides. The moisture conditions were more nearly alike on the two sides, however, and it may be that moisture plays an important part in this control, though light cannot be left out of consideration. That moisture may play an important part in the development of rhizoids was demonstrated by Dachnowski[30] in his experiments with *Marchantia polymorpha.*

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[28] Loew, O., Zur frage der Vertretbarkeit von Kaliumsalzen bei niederen Pflanzen. Bot. Centralbl. 74: 202. 1898.

[29] Prantl, K., Ueber die Anordnung der Zellen in flächenförmigen Prothallien der Farne. Flora 36: 497, 520, 545. 1878.

[30] Dachnowski, A., Zur Kenntnis der Entwicklungs-Physiologie von Marchantia polymorpha. Jahrb. wiss. Bot. 44: 254. 1907.

The number and size of the rhizoids of the gametophytes here dealt with seem to be closely related to the nutrient conditions. Fewer rhizoids were generally developed in the solution cultures than in those with soil. In solutions that were favorable to good growth, rhizoids were fewer in number, shorter and broader; while in solutions which did not favor growth, they were more numerous, longer, more slender and darker in color. Benecke [07] observed that the development of the rhizoids of Lunularia was influenced by the absorption of chemicals from the glass of the vessels in which they were cultivated. A physiological analogy would seem to exist between the development of rhizoids and the development of root hairs in higher plants. That the development of root hairs is related to the amount of water present is well known; when the roots of seedlings are grown immersed in water no root hairs or a reduced number develop. Here the cells of the root are in direct contact with water and can directly absorb it. In prothallia growing on the surface of a solution the cells are also in contact with water and direct absorption must be possible. When all the elements necessary for growth are present in sufficient quantities to meet the needs of the prothallia the stimulus for rhizoid formation appears to be weakened. If the necessary elements are not present, or the quantity is insufficient for growth, this stimulus seems to be intensified.

Adventitious outgrowths from the gametophytes were frequently developed in all the cultures. In some cases these outgrowths were mere filaments of a few cells and bore antheridia near their tips. In other cases they developed into slightly elongated prothallia with heart-shaped, notched apices and well-developed meristems bearing archegonia. Similar outgrowths have been described for other ferns by Wiegand [49], Hofmeister [51], Kny [70], Goebel [77], DeBary [78], Bauke [78], Dodel-Port [80], Klebs [93], Heim [96], Lagerberg [06], Pace [10], Heilbronn [10], and Fischer [11]. Klebs interprets the development of these outgrowths as being due to weak light. While weak light seems undoubtedly to be the cause of their origin in some cases, it cannot be considered the determining factor in all cases in the present work, for such outgrowths developed in bright light from old prothallia, or prothallia which had been transferred either from soil to solution or from one solution to another. A comparison of these results with those of previously mentioned workers indicates that, whatever factor may have been the determining one, their development was intimately connected with the vitality of the prothallia. outgrowths usually appeared when vitality was lowered, either through old age or through a shock due to unfavorable conditions.

B. SEXUAL REPRODUCTION.

The gametophyte of *Onoclea Struthiopteris* exhibits several phases of development which seem to depend largely upon environmental factors. Under conditions of poor nutrition male prothallia were developed in these studies, while under conditions of good nutrition female prothallia were in evidence. When male individuals were subjected to *favorable* nutrition there occurred a progressive development in the prothallia, shown by growth and the formation of a meristem with archegonia. If, on the other hand, female prothallia were subjected to *unfavorable* nutrition a retrogression resulted, shown by a decline in vitality and the development of antheridia. This suggests that the male tendency is associated with a lower type, and the female tendency with a higher type of development in the gametophyte. That male prothallia were developed in response to poor nutrition was shown by the solution cultures. In solutions which furnished the best cultural conditions fewer male individuals were developed and the majority of these were regular in shape. In solution cultures not as well suited to growth the proportion of irregularly-shaped males was much greater. The association of "maleness" and "femaleness" with a difference in the size and shape of the prothallia, due to unequal development, has been observed for the gametophytes of other ferns. Attention has been especially called to the "weak" development of the male gametophyte by Schacht [49], for *Pteris serrulata;* by Cornu [74], for *Aspidium filix-mas;* by Prantl [81], for Osmunda and some other ferns; by Atkinson [94], for *Niphobolus crispum corymbiferum;* by Heim [96], for *Lygodium japonicum, Balantium antarcticum, Alsophila australis,* and *Doodyacaudata;* by Life [06], for *Dicksonia apiifolia, Aneimia phyllitidis, Alsophila pruinata;* and by Lagerberg [06], for *Pteridium aquilinum.*

That the unequal development of the gametophytes is more dependent upon the factors of environment than upon inheritance is shown by the fact that most of the dioecious prothallia, when placed under different conditions of nutrition, developed the other sex. The experimental evidence here presented has clearly shown that this is true of *Onoclea struthiopteris;* not only the female prothallia, but also the males, were induced to become monoecious when placed under proper environmental conditions. Frequent reference occurs in the literature to similar observations made upon various other gametophytes. Bauke[31] speaks of an induced monoecism in *Aneimia phyllitidis,* stating that female prothallia over which moss protonema had grown developed antheridia. Also, female prothallia in cultures of *Lygodium japonicum* formed antheridia through the influence of unfavorable conditions. Prantl [81] caused "ameristic" prothallia to become monoecious by transferring them from a nitrogen-free solution to one containing nitrogen, where they developed a meristem and archegonia. Buchtien[32] conducted experiments on the gametophyte of *Equisetum pratense* and showed that when female prothallia were transferred from fertile sand to sterile sand they developed antheridia and became monoecious. Peirce [06] found that it was possible completely to suppress the development of the reproductive organs of *Gymnogramme triangularis* by insufficient light. Klebs [93] not only showed that it was possible by the reduction of light intensity to suppress the reproductive organs and keep the prothallia of *Pteris aquilina* in a vegetative stage, but that antheridia were formed when these prothallia were placed in weak light. If these prothallia were placed in stronger light archegonia were developed.

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[31] Bauke, H., Zur Kenntnis der sexuellen Generation bei den Gattungen Platycerium, Lygodium und Gymnogramme. Bot. Zeitg. **36**: 753. 1878.

Campbell[33] states that as soon as one archegonium of a gametophyte of this fern is fertilized, new ones cease to be formed. This was not the case in either my soil or solution cultures, for prothallia were observed which produced new archegonia even after fertilization had taken place and both antheridia and archegonia were produced in some cases after the young sporophyte was well developed. As in the case of the rhizoids, the shaded surface has been considered the normal one for the development of the reproductive organs. Archegonia frequently developed, however, upon the upper surfaces of female prothallia in both the soil and solution cultures. The occurrence of these organs on the upper surface can not be explained as due to the influence of light, for in the majority of cases this side was exposed to full insolation. Similar cases have been reported by Peirce [06] and others. Peirce's prothallia were grown in Knop's solution, on a clinostat and with approximately equal illumination on all sides. Here, as in the case of rhizoids, weak illumination fails to account for the development of archegonia on the upper surfaces in my cultures, for these surfaces received more light than did the under one.

A number of peculiarities were observed in the development of both antheridia and archegonia. In several cases archegonial neck cells gave rise to antheridia which appeared normal and healthy. The development of antheridia from neck cells of archegonia has been reported and figured by Miss Lyon[34] as occurring on a fern prothallium of undetermined species, probably an Osmunda. This had been reported earlier by Goebel[35] for *Hemomitis palmata* and *Lygodium japonicum*. The cases cited by Treub[36] and Hy[37] might also be considered as analogous. Treub observed an arche-

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[32] Buchtien, B., Entwickelungsgeschichte des Prothalliums von Equisetum. Dissertation. Rostock. 1878.

[33] Campbell, D. H., The structure and development of mosses and ferns. New York. 1905.

[34] Lyon, Florence M., The evolution of the sex organs of plants. Bot. Gaz. 37: 280. 1904.

[35] Goebel, K., Organographie der Pflanzen. Jena. 1898.

[36] Treub, M., Recherches sur les Lycopodiacees. Ann. Jard. Bot. Buitenzorg 3, 5, 7, 8. 1884-1890.

[37] Hy, F., Recherches sur l'archegone et le développement du fruit des Muscinees. Ann. Sci. Nat. Bot. VI. 18: 21. 1884.

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gonium of *Lycopodium phlegmaria* in which the upper canal cells divided to form a large number of cells which became spermatogenous in character. Hy observed a similar case in the mosses. Goebel [01] interpreted this general phenomenon as the result of old age. Such an explanation proves unsatisfactory for the cases observed by me, as these prothallia, like the one cited by Miss Lyon, were surely in a healthy condition. In certain other cases observed, the archegonia had developed two complete necks with but a single egg cell.

Antheridia developed on the upper as well as on the lower surfaces of the gametophytes here studied and were not restricted to any particular region of these surfaces. In some of the monoecious individuals antheridia developed among the archegonia on the meristem. Another instance of this peculiarity was observed by Miss Pace [10]. A case was also observed where two antheridia had developed upon a single stalk. This was similar in appearance to the one reported and figured by Miss Lyon.

C. SEX LATENCY.

The question of sex latency in the gametophyte generation of *Onoclea struthiopteris* is an extremely interesting one, as the genus *Onoclea* has been considered as dioecious by most writers. Campbell [87], who worked out the morphological life history of this fern in detail, emphasized "the dioecism displayed by the prothallia." That dioecism cannot be considered a marked or fixed characteristic of the prothallia of Onoclea has been clearly shown by the following lines of evidence as set forth in this investigation.

(1) Monoecious prothallia were frequently found.

(2) Monoecious prothallia occurred in cultures with various nutrient solutions.

(3) Dioecious prothallia, both males and females, were induced to become monoecious when placed under certain culture conditions.

In addition to the above evidence are the recorded observations of bisexual prothallia by Lagerberg [06], and Mottier [10].

All this evidence indicates that the sex of the gametophyte generation of *Onoclea struthiopteris* is not fixed in the spores; but that tendencies toward the production of both sorts of organs are present in the cells of the gametophytes. Therefore, there appear to be no purely male nor purely female prothallia, and Mottier's terms "purely male or female gametophytes" are hardly applicable. Among the dioecious prothallia it may be supposed that some environmental factor or combination of factors inhibits one tendency, either the male or the female, and allows the other to be effective. Thus the organisms are either male or female, as the case may be, and the other tendency is latent. In the monoecious prothallia, the influence of environmental factors or combinations of factors appears to be such that both sex tendencies are equally stimulated to become effective, and both kinds of reproductive organs are produced.

As the larger percentage of monoecious prothallia was found in the older cultures, it appears as though some physiological change took place in the cells during development, and it may be supposed that this change permits the previously latent tendency to be more easily stimulated to activity or makes this tendency less easily inhibited. The male tendency may be considered as being thus latent in all the cells of the female gametophytes; for any cell, including those which form the meristem and the archegonial necks, may produce an antheridium. This evidence supports the theory advanced by Miss Lyon [04] concerning the plasticity of the cells forming the reproductive organs. It also supports the theory, advanced by Miss Ferguson,[38] that it "might be that maleness and femaleness have no necessary relation to the form or position of sexual organs, but that the sperms and eggs are developed in relation to the sexual act or process, not as a necessary expression of sexuality."

The majority of the female prothallia which became monoecious appeared to be less healthy than those which remained female. Here it seems that monoecism was associated with lowered vitality. The latent male tendency had been stimulated to become active, through the influence of certain environmental factors, which, separately or together, also lowered the vitality of the prothallia. The failure of some female prothallia to become monoecious is not apparently due to absence of the latent male tendency, but to failure of the environment so to reduce the vitality of the cells as to call forth this tendency.

The fact that apparent differences in the vitality of the male gametophytes here studied were associated with variety of form, may indicate that the different forms of prothallia were developed in response to environmental conditions. The irregularly shaped male gametophytes, being in a poorly nourished condition, could not survive the shock of transfer from soil to solution, or from one solution to another. If this difficulty could have been overcome, so that these prothallia might have been placed under the best conditions of nutrition, there seems to be no obvious reason why they could not have developed into normal, healthy, monoecious prothallia, in the same manner as did the more or less regularly shaped males when the latter were transferred.

The effect of environmental factors upon sex differentiation in the ferns was first studied by Prantl [79]. In this work he seems to consider light as an important factor in sex control, for he had observed that strong light tended to produce only female prothallia, and weak light only male prothallia. In later work [81], the same author placed greater emphasis upon nutritive conditions, basing this opinion upon experimental evidence obtained by cultivating gametophytes on nutrient solutions. As he obtained only males on solutions from which nitrate was absent, he drew the general conclusion that an insufficient nitrogen supply might account for thickly grouped prothallia bearing only antheridia. This explanation fails, however, to account for the production of dioecious prothallia in *Onoclea struthiopteris*. In cultures made by sowing the spores upon Prantl's nutrient solution from which ammonium nitrate had been omitted, female prothallia were obtained. These were small, heart-shaped, notched prothallia with a well developed meristem bearing archegonia. The production of young sporophytes showed that these archegonia were normal and healthy. Furthermore, as has been previously stated, the position of the gametophytes in the soil cultures did not seem greatly to influence their development or sex; many of the smaller, "ameristic" prothallia, bearing only antheridia, were found in the less crowded regions of the cultures, while the larger, "meristic" ones, bearing only archegonia, were often found in more crowded regions. Monoecious prothallia were found in both regions.

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[38] Ferguson, Margaret C., Imbedded sexual cells in the Polypodiaceae. Bot. Gaz. 51: 443. 1911

Klebs' [93] work indicates that, for the particular ferns he worked with, light played a very important part in sex differentiation. Later work by Heim [96] and Peirce [06] points to the same conclusion. While we must admit that light may be an important factor, we must not lose sight of the possible importance of other factors of the environment. Even by cultivating prothallia upon nutrient solutions whose chemical constituents are known, we are far from having determined the effects of the chemical reactions which may result from the presence or absence of the different chemical elements. Our only hope of ever gaining such knowledge lies in further experimental study of solution cultures under known conditions of light and temperature.

Klebs has demonstrated that an intimate relation exists between growth and reproduction. Since growth is dependent upon nutrition, then nutrition is the most important factor both in growth and reproduction. It is a well known fact that growth can be carried on under conditions of nutrition which do not prove sufficient for reproduction. In the production of the egg cells and their necessary accessories a greater demand is made upon the prothallia than results from the vegetative processes alone. In order to meet this demand greater growth must precede reproduction. The larger heart-shaped prothallia are able to meet this demand, having had a more vigorous growth, and hence they are able to develop female reproductive organs.

Viewed in the light of the plasticity of plant bodies in relation to their environmental factors, it seems that sex is not predetermined in the spore of *Onoclea struthiopteris,* but that tendencies toward the production of both kinds of sex organs exist as potentialities in the cells of the prothallia, and are influenced by environmental factors to become active or to remain latent. These controlling factors are not only effective during the early development of the gametophyte but may continue so during the greater part of its life history.

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A QUANTITATIVE CHEMICAL AND PHYSIOLOGICAL STUDY OF NUTRIENT SOLUTIONS FOR PLANT CULTURES.[1]

WILLIAM E. TOTTINGHAM.

ABSTRACT.[2]

The purpose of this investigation was to study the influence, upon the growth of young wheat plants, of a wide range of proportions of the component salts in nutrient solutions and the alterations of this influence produced by different total concentrations. Preliminary to the chief part of the investigation, methods were developed for preparing Knop's nutrient solution more accurately than is usually the case. The concentration limiting stability of this solution at temperatures ranging from about 20° to about 23° C. was determined both when di-potassium phosphate and when mono-potassium phosphate were employed as the source of phosphorus. In Knop's solution with concentrations sufficiently high to cause precipitation, the calcium, phosphorus and sulphur contents of the supernatant solutions were determined. Wheat seedlings, started in water, were grown 24 days in Knop's solutions of various concentrations (both with mono- and with di-potassium phosphate as source of phosphorus) to determine approximately the optimum concentration of this solution for this period of growth of the plant. By aid of published data of electrolytic dissociation and of osmotic pressure, the osmotic pressure of Knop's solution was approximately calculated for a wide range of total concentrations. Three different total concentrations were employed in the main part of the study, one optimum, the second too low and the third too high for the best growth of the young wheat plants. These concentrations were 0.60, 0.01 and 2.00 per cent., having approximately 2.50, 0.05 and 8.15 atmospheres of osmotic pressure, respectively. In each of these cases the approximate osmotic pressure was divided into ten equal parts and these ten parts were distributed among the four component salts of Knop's solution (using mono-potassium phosphate as source of phosphorus) in all possible ways, thus giving eighty-four solutions for each series, all of these solutions having approximately the same osmotic pressure, but no two of them containing the same relative proportions of the four salts. Yields of roots and of tops, as well as various other quantitative data on the growth

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of the plants in these three series of eighty-four cultures each, are presented, partly by aid of triangular diagrams or graphs. Finally, the evaporating power of the air was measured during the growth of each series, by means of a Livingston porous cup atmometer and the relative water losses of selected cultures from each isosmotic series were determined by weighings for short periods just preceding harvest.

The chief results are as follows:

1. It was found convenient to employ the component salts of Knop's formula in stock solutions, one part (A) containing only calcium nitrate and the other part (B) containing the remaining three salts.

2. The maximum possible concentration, without precipitation, of part B, for a period of 14 days at temperatures from 20° to 23° C., was found to be 10.0 per cent. when mono-potassium phosphate was employed as the source of phosphorus, and 9.0 per cent. when the di-potassium salt was similarly used. These values limit the concentrations of the two forms of part A to 12.33 and 12.0 per cent. respectively.

3. The maximum possible concentration of the complete Knop's solutions, without precipitation, for a period of from three to four days at the temperatures above mentioned, was found to be 2.2 per cent. and 2.8 per cent. when K_2HPO_4 and KH_2PO_4 were employed, respectively.

4. It was found that the precipitate formed in unstable Knop's solutions consists almost entirely of $CaSO_4 \cdot 2H_2O$.

5. Methods for germinating seeds and for mounting the seedlings in culture solutions are described, which differ somewhat from those heretofore employed; the most important modification being the arrangement of a two piece stopper for the culture jars, to hold six seedlings.

6. Preliminary cultures of wheat in two forms of Knop's solution, one including mono- and the other di-potassium phosphate, produced nearly equal dry weights of tops through a range of concentration of the medium from 0.05 to 0.80 per cent., the concentration varying by increments of 0.05 per cent.

7. In these cultures mono-potassium phosphate produced 17.8 per cent. better growth of tops and 17.5 per cent. better growth of roots than did the di-potassium salt, on the basis of yields from these two forms of solution relative to their respective control cultures in distilled water alone.

8. Of the osmotically equivalent culture solutions of *0.01 per cent. concentration* (approximately 0.05 atmosphere of osmotic pressure) the solution producing the greatest dry weight of tops was 39.0 per cent. superior to Knop's solution of the same concentration, on the basis of the yields of these two solutions relative to their respective control cultures in distilled water. The volume-molecular partial concentrations (gram molecules per liter of solution) of KH_2PO_4, $MgSO_4$, $Ca(NO_3)_2$ and KNO_3 in

this superior solution were .000065 *m*, .000378 *m*, .000187 *m* and .000189 *m*, respectively. This solution thus contained 0.6, 3.1, 0.5 and 1.4 times as much of the respective salts (in the order above given) as occurs in the corresponding Knop's solution. In the case of roots the solution producing the greatest dry yield in this series was 15.4 per cent. superior to Knop's solution of the same total concentration, on the same basis as that stated above. The volume-molecular partial concentrations of the four salts in this solution, following the same order as before, were .000194 *m*, .000094 *m*. .000062 *m* and .000472 *m*, respectively, this solution thus containing 1.8, 0.8, 0.2 and 3.4 times as much of the respective salts as occurs in the corresponding Knop's solution.

9. The solution producing the greatest dry weight of tops in the series with *0.6 per cent. total concentration* (approximately 2.5 atmospheres of osmotic pressure) was 11.0 per cent. superior to Knop's solution of the same concentration, on the basis already employed. The volume-molecular concentrations of the four salts in this superior solution, in the order previously adopted, were .0130 *m*, .0145 *m*, .0144 *m* and .0049 *m*, respectively, this solution having 2.1, 2.1, 0.7 and 0.6 times as much of the respective salts as occurs in the corresponding concentration of Knop's solution. The solution producing the greatest dry weight of roots in this series was 29.0 per cent. superior to the corresponding Knop's solution, on the basis already employed. The volume-molecular concentrations of the four salts in this solution, in the adopted order, were .0208 *m*, .0058 *m*, .0036 *m* and .0195 *m*, respectively. This solution had 3.3, 0.8, 0.2 and 2.3 times as much of the respective salts as is contained in the corresponding Knop's solution.

10. The solution producing the greatest dry weight of tops with *2.0 per cent. total concentration* of the medium (approximately 8.15 atmospheres of osmotic pressure) was 53.0 per cent. superior to Knop's solution of the same total concentration, on the basis already stated. This superior solution contained the following volume-molecular partial concentrations of salts, in the adopted order: .0168 *m*, .0595 *m*, .0180 *m* and .0734 *m*, respectively, or 0.8, 2.5, 0.3 and 2.7 times as much of the respective salts as occurs in the corresponding Knop's solution. Similarly, the solution producing the greatest dry weight of roots was 47.0 per cent. superior to Knop's solution. This solution contained the following volume-molecular partial concentrations of the four salts: .0169 *m*, .0895 *m*, .0240 *m* and .0408 *m*, respectively. These values represent 0.8, 3.8, 0.3 and 1.5 times as much of the respective salts as do the corresponding values for Knop's solution of the same total concentration.

11. The most important factor affecting the physiological influence of varying proportions of the four salts in these culture solutions was apparently the ratio of the magnesium content to that of calcium. In the cultures

producing the greatest dry weight of tops in the three series of osmotically equivalent culture solutions (with 0.01, 0.60 and 2.00 per cent. concentration, respectively) the ratios of magnesium to calcium were, respectively, 2:1, 1:1, and 3.3:1.0, while the corresponding ratio in Knop's solution is 1:3.

12. The solutions containing the highest proportions of $MgSO_4$ depressed the growth of tops slightly in 0.01 per cent. concentration, produced characteristic and serious injury in 0.6 per cent. concentration and caused more generally prevalent but less serious injury in 2.0 per cent. concentration. High proportions of $Ca(NO_3)_2$ in the series of solutions with 2.0 per cent. concentration produced characteristic and serious injury to the tops.

13. The ratio of rate of water loss to dry weight of tops, for selected cultures from these three chief series of solutions (concentrations of 0.01, 0.60 and 2.00 per cent., respectively) ranged from 1.0 to 1.5, from 1.0 to 1.9 and from 1.0 to 6.0 for the respective series. In the last of these series greatly suppressed transpiring power accompanied excessive injury due to magnesium.

14. It is shown that the injurious effect of magnesium and the amount of dry matter produced by the plants depends upon the complex balance between all of the salts in solution. Furthermore, the effects upon growth, of any given set of proportions of the nutrient salts in the medium are shown to vary with the total concentration of the latter.

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PART I.

HISTORICAL SURVEY OF EXPERIMENTATION WITH PLANTS IN WATER CULTURES.

INTRODUCTION.

The method of water cultures, or solution cultures, which eliminates many of the complex factors influencing the growth of plants in soil, has been recognized for a long time as presenting favorable conditions for studying the chemical requirements of plants. It has been of special value in distinguishing the essential from the non-essential chemical elements occurring in these organisms; and more recently it has been of great service also in investigations dealing with the tolerance of certain plants for various toxic salts occurring in alkali soils, with the effects of certain organic constituents of the soil upon plant growth and with the selective power of the plant for the constituent ions of various inorganic salts.

No attempt is made here to give a complete review of the very extensive literature covering the subject of solution cultures. In collecting the references of the following section, *Die Landwirtschaftliche Versuchsstationen*, which contains most of the earlier publications in this field, has been thoroughly reviewed, and the most important researches have been presented. Such references have been added as seemed to contribute toward a connected account of the development of the subject. For a brief general treatment of this matter the works of Czapek,[3] Pfeffer[4] and Duggar[5] may be consulted.

REVIEW OF LITERATURE BEARING ON THE PRESENT WORK.

The early employment of water cultures was for purposes of morphology rather than for those of physiology, attention being attracted first by the favorable conditions thus offered for the observation of the structural development of root systems. Nevertheless, in the earliest recorded experiments with this method of culture, Woodward[6] undertook to discover whether it was the water or the solid particles of the soil which nourished plants. He grew spearmint, potato and vetch, for periods in some cases as long as 77 days, and employed rain, spring, river, conduit and distilled waters. He concluded that water was not a nutrient substance, but was a carrier of "terrestrial matter" from which the plant was formed and nourished.

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[3] Czapek, F., Biochemie der Pflanzen 2: 838-40. Jena, 1905.

[4] Pfeffer, W., The physiology of plants, translated by A. J. Ewart 1: 418-22. Oxford. 1900.

[5] Duggar, B. M., Plant physiology. New York. 1911. Pages 142-50.

[6] Woodward, J., Thoughts and Experiments on Vegetation. Phil. Trans. Roy. Soc., London, 21: 382-98. 1699.

Previous to 1750, Duhamel du Monceau[7] conducted water cultures with the bean, walnut, almond and chestnut, germinating the seeds in moist sponges and growing the plants in filtered water from the Seine river. The bean plants grew to maturity and the trees survived for several years, the oak attaining a height of 18 inches in eight years. He observed indications that some part of the soil or fertilizers entered the plant. From the products obtained by distillation of plants raised in soil and in water cultures he concluded that the plant had the same composition in both cases.

About 1800, de Saussure[8] investigated the absorption of certain salts and organic substances by the roots of bog plants. He grew *Polygonum persicaria* (knotweed) and *Bidens cannabina* (beggar ticks) for five weeks, in various solutions each containing from one to three of the compounds studied. His conclusions, sometimes referred to as his "laws," were chiefly these: that roots extract less of salts than of water from a given solution, and that all the compounds in a solution are not equally absorbed, those forming solutions of lowest viscosity being absorbed in the greatest quantities.

For a considerable period this method of culture received little attention, but for the decade beginning about 1860 Knop, Nobbe, Sachs and several other workers were especially active in the study of optimum conditions for conducting water cultures, and devoted attention to the kind, form and proportions of the chemical elements supplied and to the concentration of the nutrient solutions.

In 1859 Knop and Sachs employed water cultures in their first nutrition experiments. The former[9] investigated by this means Liebig's idea that the constituents dissolved in soil water are not generally sufficient for plant growth and that plants derive food directly from the soil particles. Knop also studied the problem as to whether or not transpiration controls absorption. He germinated dwarf kidney beans in water and grew the plants for 82 days in spring water. His analyses of both seeds and plants demonstrated that absorption had occurred. He also grew the plants in salt solutions, to which, in some cases, humus preparations or organic acids had been added or from which ammonia or nitrates had been omitted. Sachs[10] used this method to determine the influence of chemical and physical effects of the soil on the transpiration of plants. He grew maize, tobacco and pumpkin in solutions of single salts and acids and in complete nutrient solutions, germinating the seeds in moist sawdust. In this manner he grew Indian corn to the second generation. This writer concluded that the roots of land plants can grow normally in water, and that if the solution is properly balanced the plants may produce viable seeds; also, that increase in the concentration of a nutrient solution diminishes the length of roots but does not otherwise alter either their morphological characters or their specific capacity to supply water and salts to the plant.

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[7] Duhamel du Monceau, H. L., La physique des arbres **2**: 202-4. Paris. 1758.

[8] de Saussure, Th., Recherches chimiques sur la végétation. Paris. 1804. Chapter 8.

[9] Knop, W., Ein Vegetationsversuch. Landw. Versuchsst. **1**: 181-202. 1859.

[10] Sachs, J., Ueber den Einfluss der chemischen und physikalischen Beschaffenheit des Bodens auf die Transpiration der Pflanzen. Landw. Versuchsst. **1**: 203-40. 1859.

——— Erziehung von Landpflanzen in Wasser. Landw. Versuchsst. **2**: 22-31. 1860.

Knop[11] carried out further work bearing upon Liebig's theory, as above mentioned, and studied the function of the ash constituents of plants and the metabolic processes. He pointed out the problems as to the best qualitative combinations of the basic and acid elements recovered in plant ash and the best relative quantities of these combinations to be employed for nutrient solutions. In his earlier experiments the seeds were germinated in a shallow layer of water until the radicles were about 2.5 cm. long, and then from twenty to thirty seedlings were placed on gauze or netting stretched over culture jars containing a liter of analyzed spring water. When the stems were just appearing the nettings were transferred to jars containing various amounts and combinations of KNO_3, KH_2PO_4, $Ca(NO_3)_2$ and K_2SO_4, in spring water. This author studied changes of concentration of the nutrient solution, conduct of the plant with reference to excretion, when changed from a salt solution to distilled water, and the influence of organic substances added to the culture solution. Beans, maize, winter rape and tobacco were grown from seed and Tradescantia from cuttings, and the total yields by weight, relative to the original weight as unity, were found to vary from 1.75 for the bean to more than 7.0 for Tradescantia. A slight excretion of potassium phosphate, occurring when the plants were transferred from a solution of that salt to distilled water, was taken as evidence that roots can attack the soil particles. Knop's conclusions, of pertinence here, were: that KNO_3 and KH_2PO_4 together can produce growth but not normal fruiting, that the net result of absorption is the attainment of equal concentration in the cell sap and in the residual nutrient solution (the salts seeming to be absorbed unaltered in composition), that exosmosis occurs if the concentration of the external solution is less than that of the plant sap, and that organic matter in the nutrient medium does not benefit the plant. The general conclusion from these extensive investigations was that, while water-culture plants were not normal, the results justified further investigation.

In 1860 Sachs[12] proposed the first standard formula for a nutrient solution for plants. He believed the method of water culture to be valuable because it eliminated adhesion, absorption and other effects of solid media such as the soil, but concluded that it was impossible to prepare a nutrient solution containing the requisite quantities of the essential acids and bases simultaneously, on account of precipitation, such as of calcium and iron phosphates. His solution consisted of two parts. Part A (without phosphorus) contained KNO_3, $NaCl$, $CaSO_4$, $MgSO_4$, $FeSO_4$ and $MnSO_4$; while Part B contained Na_2HPO_4, KNO_3, $NaCl$, K_2SO_4 and Na_2SO_4. Maize, bean and beet were grown by Sachs for periods up to 116 days in length. In some cases only one part of his solution was employed and in others the same plants were grown alternately in parts A and B. The compositions of the nutrient solutions employed varied during the growth of a given culture and the concentrations ranged from 0.1 to 0.4 per cent. The so-called "normal" solution of Sachs, from which dilutions were made for the last stage of his culture experiments, was a 4.5 per cent. solution consisting of 2 per cent. KNO_3, 1 per cent. Na_2SO_4, 1 per cent. $MgSO_4$ and 0.5 per cent. $NaCl$. Iron was supplied to the cultures in the form of $FeSO_4$.

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[11] Knop, W., Ueber die Ernährung der Pflanzen durch wässerige Lösungen bei Ausschluss des Bodens. Landw. Versuchsst. **2**: 65-99, 270-93. 1860.

[12] Sachs, J., Vegetationsversuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernährungsbedingungen von Mais, Bohnen und anderen Pflanzen. Landw. Versuchsst. **2**: 219-68. 1860.

Maize was grown to maturity from the seedling stage, each jar of 1500 cc. holding five plants. The nutrient solution was changed on the 55th, on the 64th and on the 81st day; and larger jars were substituted at the last change. A harvest of grain was obtained, weighing more than eighteen times as much as the original seed. In another experiment the ratio of the average daily growth of such cultures to that of plants grown in garden soil, was 0.77. Sachs concluded that Indian corn could grow normally in solution cultures with proper concentration of the requisite salts (the optimum total concentration appearing to be about 0.3 per cent.); that specific physical properties of the soil were not essential to growth, and that, with this plant, elements deficient in the nutrient solution at earlier stages might be advantageously absorbed later, even up to the time of fruiting. From cultures in the solution containing the other essential elements, he concluded that calcium phosphate (probably referring to $Ca_3(PO_4)_2$) could serve as the source of calcium and phosphorus up to the time of flowering.

In 1861 Knop[13] reported the results of extensive investigations with water cultures, to determine the relative quantities of the essential nutrient elements absorbed by the plant. Maize, barley, oats and other plants were employed, the original plan being to maintain a constant composition of the nutrient solution between changes of solution, but to increase the concentration of the latter at each change; the periods between changes were marked by the transpiration of one liter of water. It was found necessary, however, to double and finally to triple the amount of PO_4 employed.

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[13] Knop, W., Quantitativ-analytische Arbeiten über den Ernährungsprocess der Pflanzen. Landw. Versuchsst. 3: 295-324. 1861.

The nutrient solution for this work consisted of a mixture, in molecular proportions, of one part of $MgSO_4$, two parts of $Ca(NO_3)_2$ and two parts of KNO_3. To 500 cc. of this solution were added 0.1 g. of freshly precipitated $FePO_4$ and from 10 to 30 cc. of a solution containing 0.01 g. KH_2PO_4 per cubic centimeter. The so-called " normal " solution of Knop, consisting of the first three salts just mentioned, contained, for a volume of 500 cc., the following amounts of basic and acid ions[14]: 1.0800 g. NO_3, 0.2070 g. $SO4$, 0.2440 g. Ca, 0.0700 g. Mg and 0.3900 g. K, making a total salt content, as thus expressed, of 0.0810 g. Thus the 510 to 530 cc. of resulting solution contained 2.2810 to 2.4810 g. of total dissolved salts, corresponding to a concentration of from 0.45 to 0.47 per cent. (grams per 100 cc. of solution).

Knop found that potassium and calcium were absorbed from these cultures in the proportions supplied, but that only from one-third to one-half of the magnesium present was taken up, and concluded that the plant influences the composition of the nutrient medium by excretion or selective absorption. He recommended the molecular proportions of salts given in the two following formulae, suggesting that B might contain all the essential elements

A 1 $MgSO_4$, 4 $Ca(NO_3)_2$, 4 KNO_3 and x KH_2PO_4.[15]
B 1 $Mg(NO_3)_2$, 4 $Ca(NO_3)_2$, 4 KNO_3 and x KH_2PO_4.[15]

In a continuation of the preceding work, Knop[16] found that concentrated solutions retarded transpiration. He also concluded, from the extent to which various elements were absorbed by the plant, that there was less resistance of the plant membranes to the passage of nitrate than to that of sulphate, thus introducing the conception of protoplasmic permeability as a factor in the absorption of nutrient elements. He further found that silicon was unessential for the plants used, that excess of nitrate was taken up undecomposed, that there was an excess of sulphate in his media, and that all the phosphate had been removed from solution at each change of the latter. Finding that calcium was taken up to a greater extent than magnesium, he believed that $MgSO_4$ was dissociated and that the anion SO_4 was absorbed by the plant in larger amount than the cation. As a result of this investigation he favored formula A of his preceding paper.

In 1862 Nobbe and Siegert,[17] took up the question whether or not chlorine is a specific nutrient for plants. As the basis for their culture solutions they used Knop's [61] formula B, mentioned above, and found that buckwheat could develop in this solution so as to give a crop having a dry weight 200 times that of the original seed.

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[14] The author does not give these data in the form here employed; recalculation from his figures has been resorted to in order to bring them into modern form.

[15] x is here used to denote that the amount of the salt in question was not definite. The same notation will be employed for similar cases in the sequel.

[16] Knop, W., Quantitativ-analytische Arbeiten über den Ernährungsprocess der Pflanzen. II. Landw. Versuchsst. **4**: 173-87. 1862.

[17] Nobbe, F., and Siegert, T., Ueber das Chlor als specifischen Nährstoff der Buchweizenpflanze. Landw. Versuchsst. **4**: 318-40. 1862.

Stohmann[18] carried out experiments with maize, at about this time, to determine whether nutrient solutions could produce greater growth than that produced by the seed and water alone. He based his solution formulae upon the composition of the ash of the grain and of the stover of this plant, employing his salts in the proportion of one part of the former to fifty parts of the latter. He employed two solutions, one (A) containing KNO_3, K_2SiO_3, and Na_2HPO_4, and the other (B) $MgSO_4$, $FeCl_3$ and $Ca(NO_3)_2$. Various other solutions were prepared to omit certain elements or to supply nitrogen exclusively in one compound. This author observed[19] that the growth of the plants rendered the solution alkaline, with production and precipitation of FeS. Thus he observed, apparently unwittingly, selective absorption.

An investigation of the optimum concentration for a nutrient solution was reported by Nobbe and Siegert,[20] in which seedlings of barley and buckwheat, germinated in folds of moist blotting paper, were placed in liter glass cylinders of nutrient solution. The nutrient solution used was a modification of Knop's solution, designated later in the literature as "Nobbe's solution," and consisted of the following molecular proportions of salts: 1 $MgSO_4$, 4 $Ca(NO_3)_2$, 4 KCl, 0.1 $FePO_4$, 0.25 KH_2PO_4, the last two salts being added periodically, in small amounts, during the progress of the experiment. The total salt concentrations used ranged from 0.05 to 1.0 per cent. It was noted that in low concentrations (of 0.05, 0.1 and 0.2 per cent.) long and thickly haired roots developed, while in solutions approaching the highest concentration used, the main roots were short and the laterals and root hairs poorly developed. These writers found the length of the period of the life cycle nearly proportional to the total salt concentration of the nutrient solution employed, and placed the optimum strength of the latter at 0.3 per cent. of total salts.

In this same solution Nobbe[21] grew a successful culture of the potato. He concluded that it was important to renew the nutrient solutions to avoid the accumulation of excreted physiological products and advised the use of a concentration of 0.05 or 0.1 per cent. and the employment of large culture jars or the frequent renewal of the solutions. The latter recommendation aimed to simulate the absorptive effects of the soil, by which accumulation of excreted material was supposed to be prevented. Of considerable interest is his further observation, that buckwheat required a higher minimum and endured a higher maximum concentration of the nutrient solution than did barley, under otherwise similar conditions.

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[18] Stohmann, F., Ueber einige Bedingungen der Vegetation der Pflanzen. Ann. Chem. und Pharm. **121**: 285-338. 1862.

[19] Stohmann, F., Vegetationsversuche in wässeringen Lösungen. Landw. Versuchsst. **4**: 65-7. 1862.

[20] Nobbe, F., and Siegert, T., Beiträge zur Pflanzencultur in wässerigen Nährstofflösungen. I. Ueber die Concentration der Nährstofflösungen. Landw. Versuchsst. **6**: 19-45. 1864.

[21] Nobbe, F., Die Kartoffel als Wasserpflanze. Landw. Versuchsst. **6**: 57-61. 1864.

In the same year Nobbe and Siegert[22] reported a further investigation of chlorine as a nutrient for buckwheat in water cultures. Their solutions had a concentration of from 0.1 to 0.3 per cent. of total salts. The physiological significance of chlorine first became noticeable at the fruiting stage, when it appeared that KCl and $CaCl_2$ were superior to NaCl and $MgCl_2$ as sources of this element.

Stohmann[23] grew the potato to partial maturity in nutrient solutions containing total salts in a concentration of 0.3 per cent. His two solutions had the following composition, in molecular proportions:

A 1 $MgSO_4$, 4 $Ca(NO_3)_2$, 2 NH_4NO_3 and 3 KH_2PO_4[24] ("$KOPO_5$"), 1/3 $FeCl_3$ and x K_2SiO_3.

B 1 $MgSO_4$, 4 $Ca(NO_3)_2$, 2 KNO_3, 1 KH_2PO_4[24] and 1/3 $FeCl_3$.

Tubers were formed in sixty days and, although they were mostly very small at the time of harvest, some weighed as much as 20 g. Stohmann grew maize also in his solution B and in various modifications of this, replacing daily the water lost by transpiration, adding KH_2PO_4 from time to time and renewing the solution three times during the period of the experiment. The best results were obtained from formula B as given above.

In 1864 Wolf[25] entered the field of water culture experimentation with an investigation of salt absorption by maize and the bean. He employed solutions containing two salts with a common anion or cation, to test de Saussure's law of absorption. He found that the bean absorbed greater absolute amounts of $Ca(NO_3)_2$, $CaSO_4$ and KH_2PO_4, but less KNO_3, than did Indian corn. In very dilute solutions, below a concentration of about 0.05 per cent., the plants absorbed a greater weight of salts than of water, thus failing to uphold de Saussure's law, which states that assimilation of salts is proportional to the concentration of the nutrient solution. At higher concentrations assimilation was about proportional to the concentration of the medium and the residual solutions were not appreciably altered in composition. Wolf concluded that transpiration was influenced by the concentration of the solution and by the permeability of the plant surfaces to salts. He classified salts as easily and difficultly diffusible, and concluded that magnesium was very toxic to the plants used in his work.

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[22] Nobbe, F., and Siegert, T., Beiträge zur Pflanzencultur in wässerigen Nährstofflösungen. II. Ueber das Chlor als Pflanzennährstoff. Landw. Versuchsst. **6**: 108-20. 1864.

[23] Stohmann, F., Vegetationsversuche in wässerigen Lösungen. Landw. Versuchsst. **6**: 347-55. 1864.

[24] Stohmann's formula for this salt is given in the parenthesis.

[25] Wolf, W., Die Saussure'schen Gesetze der Aufsaugung von einfachen Salzlösungen durch die Wurzeln der Pflanzen. Landw. Versuchsst. **6**: 203-30. 1864.

Further work was contributed at this time by Rautenberg and Kühn,[26] who studied the possibility of replacing NO_3 by NH_3 and the importance of $FeSO_4$ and SiO_2 in the nutrition of plants. Windsor bean and maize were grown in various solutions having a concentration of 0.3 per cent. of total salts, based upon the following two standard solutions, the proportions being molecular:

A 1 $MgSO_4$, 2 $Ca(NO_3)_2$, 4 KNO_3, x KH_2PO_4.
B 1 $Mg(NO_3)_2$, 4 $Ca(NO_3)_2$, 4 KNO_3, x KH_2PO_4.

The solutions were renewed several times during each experiment. A rapid production and increase in the acidity, which these writers attributed to selective absorption of NH_3, occurred when NH_4Cl was employed, but was not marked when the nitrate, sulphate or phosphate of ammonium was used. Rautenberg and Kühn concluded that iron, sulphur and silicon were essential elements for the growth of these plants, and obtained a greater yield of grain when nitrogen was supplied as nitrate than when it entered the solution as an ammonium salt.

In 1865 Knop,[27] as a result of what must be regarded as his most important investigation, proposed what is now recognized as one of the standard formulae for nutrient solutions. This resulted from an extensive continuation of his previous study of the quantitative relations involved in the absorption of salts by plants in nutrient solutions. Wheat, oats, barley and rye were grown separately, three plants being placed in a liter jar when the plants were about 15 cm. high, and the cultures were continued for 49 days after this transfer. Ten different solutions, containing various proportions of four nutrient salts and varying in total concentration from 0.030 to 0.325 per cent. were employed, and these were renewed after periods of from two to eight days in the various series. Knop concluded that no nutrient solution was possible that might be assimilated unaltered, but that there might be a most favorable solution for plant cultures, and proposed as the best formula, suitable for grasses and buckwheat, the following: 0.1 per cent. $Ca(NO_3)_2$, 0.025 per cent. KNO_3, 0.025 per cent. KH_2PO_4, 0.025 or 0.0125 per cent. $MgSO_4$ and a small quantity of $FePO_4$. He believed it might be advantageous to add calcium phosphate. Knop further concluded that these salts, in addition to water and carbon dioxide, were the only essential plant nutrients, and that NH_4, Si, F, Cl, humus, etc., while they might be beneficial, were not to be regarded as necessary for growth.

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[26] Rautenberg, F., and Kühn, G., Vegetationsversuche im Sommer 1863. Landw. Versuchsst. 6: 355-9. 1864.

——— Berichte über die auf der landwirthschaftlichen Versuchsstation Weende ausgeführten Versuche. XVII. Vegetationsversuche im Sommer 1863. Jour. Landw. 12: 107-44. 1864.

[27] Knop, W., Quantitative Untersuchungen über den Ernährungsprocess der Pflanze. Landw. Versuchsst. 7: 93-107 1865.

Nobbe,[28] in discussing experimental methods for water cultures, remarked that solutions containing from 0.05 to 0.1 per cent. of salts (renewed more or less often according to the size of plants and culture jars) gave early-maturing, normal plants, and suggested the desirability of a circulating or continuously renewed nutrient solution to maintain the original concentration.

In a quantitative study of absorption from solutions of two salts by plants of bean and maize, Wolf[29] concluded that this process is controlled by the external concentration in solutions containing more than 0.25 per cent. of salts, but that below this concentration absorption is controlled by the salt concentration in the roots and by the influence of one salt upon another in the external solution. Finding that only traces of magnesium, calcium and SO_4 were excreted from the roots of plants during several days after transfer from nutrient solutions to distilled water, he concluded that absorbed salts are held in the plant cell in a non-diffusible condition, and that this apparent affinity of the cell contents for salts is an appreciable factor in absorption. Wolf assumed that the nutrient salts were decomposed, after absorption, by assimilation or metabolic changes; he also pointed out the applicability of water cultures to soil problems.

Leguminous plants were first employed extensively in water cultures by Lucanus[30] who grew clover, vetch, lupine, pea and beet in a solution having a total concentration of 0.3 per cent., the component salts being in molecular proportions, as follows: 2 $Ca(NO_3)_2$, 1 KH_2PO_4, 0.1 $MgSO_4$ and x $FePO_4$, with various modifications that introduced the elements, chlorine, sodium, calcium, rubidium and lithium. He concluded that the molecular ratio of calcium to potassium in the medium should be 2:1 for the best growth of clover, and his yields indicated the possibility of a partial replacement of potassium by sodium and rubidium.

In the same year Nobbe[31] reported further work on the importance of chlorine, following his earlier methods, already outlined. In this work concentrations of 0.05 and of 0.1 per cent. were employed.

In 1866 Birner and Lucanus[32] published the results of the most elaborate series of water cultures conducted up to that time. They germinated oats in sawdust extracted with hydrochloric acid, and grew the plants to maturity in Stohmann's and Knop's solutions. The chief subjects investigated were: (1) the influence of the concentration of the nutrient solution; (2) the influence of sodium, chlorine, silicon and manganese; (3) the relative requirements by plants for the constituents of a "normal" nutrient solution; (4) the relative effectiveness of various nitrogen compounds; (5) the relative effects of oxides and sub-oxides of the elements, and (6) the vitality of the seed produced in water cultures. The "normal" nutrient solution used in these investigations had a concentration of 0.3 per cent. and contained the following salts, in chemically equivalent proportions: 0.01 $MgSO_4$, 0.02 $Ca(NO_3)_2$, 0.01 KH_2PO_4 and 0.001 $FePO_4$.

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[28] Nobbe, F., Die Züchtung der Landpflanzen in Wasser betreffend. Landw. Versuchsst. 7: 68-73. 1865.

[29] Wolf, W., Chemische Untersuchungen über das Verhalten von Pflanzen in der Aufnahme von Salzen aus Salzlösungen, welche zwei Salze gelöst enthalten. Landw. Versuchsst. 7: 193-218. 1865.

[30] Lucanus, B., Versuche über die Erziehung einiger Landpflanzen in wässerigen Lösungen. Landw. Versuchsst. 7: 363-71. 1865.

[31] Nobbe, F., Ueber die physiologische Function des Chlor in der Pflanze. Landw. Versuchsst. 7: 371-86. 1865.

[32] Birner, H., and Lucanus, B., Wasserculturversuche mit Hafer in der Agrik.-chem. Versuchsstation zu Regenwalde. Landw. Versuchsst. 8: 128-77. 1866.

To allow recovery of the roots from injury occasioned by transfer of the plants from the germination medium, the seedlings were first placed for a time in distilled water. Finally they were placed, one plant for each single liter of solution, in 5- and 6-liter jars with shellacked zinc covers. These jars stood on tiers of shelves in a shaded greenhouse and control plants were grown simultaneously in soil. Water was replaced or solution added daily, to offset that lost by transpiration, and in about 55 days the solutions were renewed to prevent alkalinity. Increased growth was obtained by conducting CO_2 through the nutrient solutions. Although considerably better growth was exhibited with a solution having a concentration of 0.3 per cent., these authors concluded that 0.1 per cent. was favorable, and also that the relative amounts of the different salts in the nutrient solution may vary widely without appreciable effect upon growth. They further concluded that sodium, silicon, chlorine and manganese were unessential, that SO_2 and NO_2 were toxic and that potassium could not be replaced by closely related elements, nor NO_3 by either NH_4 or organic compounds of nitrogen.

At about the same time Wolf[33] reported experiments with maize, oats, beans and clover, grown in a nutrient solution prepared by decomposing bone ash with nitric acid, neutralizing with K_2CO_3 and adding nutrient salts. The culture vessels had a capacity of 1700 cc., in the beginning of the experiments, but ones of double this size were used later. The longest period of growth, 88 days, occurred with beans. Changes of solutions were at first made every ten days, later every seven days. Wolf also investigated the solution employed by Nobbe [62] for the growth of buckwheat. He concluded that the concentration of total salts of from 0.1 to 0.2 per cent. was the best for Indian corn and wheat, and that the proportion of NO_3 should be relatively high.

At this stage in the development of the water culture method interest seems to have centered about the question of the availability of nitrogen in organic compounds, particularly in those of animal urine. Among others, Hampe[34] conducted experiments upon maize with this question in view, employing urea and uric acid in nutrient solutions.

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[33] **Wolf, E. T., Ueppige Vegetation in wässerigen Lösungen der Nährstoffe. Landw. Versuchsst. 8: 189-215. 1866.**

In a general discussion of his earlier work Nobbe[35] described a method for solution cultures involving a circulating nutrient solution. He had devised this in 1865 to avoid the detrimental effects of increased concentration and disturbed balance of the elements in the solution brought about by transpiration. He arranged a siphon to deliver his standard nutrient solution drop by drop, from a 10-liter stock supply into the bottom of a 2-liter culture flask, a constant level being maintained by overflow. By this arrangement he grew buckwheat to maturity in a 0.1 per cent. solution and concluded that this was the best concentration to employ. In this investigation Nobbe introduced what appears to have been the first use of meteorological data in defining the aerial conditions under which the experiments were carried out.

In 1867 Nobbe[36] reported experiments with biennial plants, showing that these could mature in water cultures during the second year, if carried through the winter in sand. He grew rape and beet in his standard nutrient solution (see page 143 above, Nobbe and Siegert [64, *1*]) except that he here used only one-fourth as much $MgSO_4$ as this standard requires.

After 1874, for a period of several years, little was done with water cultures, but in the succeeding decade the method was revived to a considerable extent. There may be mentioned for the present purpose only the more important investigations which included the work of Jodin,[37] Kellner,[38] and Kreuzhage and Wolf.[39] Of marked importance was Kellner's conclusion that ammonia and nitrate together in the same solution produced a better effect upon swamp rice than did either alone.

During the decade following 1885 there was another period of comparative inactivity in this general field of investigation. Of the work with water cultures from 1898 to the present, mention will be made below of some of the more important and characteristic researches.

Dassonville[40] used Knop's [65] solution A and various other salt solutions in a study of the morphology and histology of several plants grown in water cultures. He found that magnesium sulphate alone retarded growth, while potassium phosphate was indispensable. Potassium favored growth and increased the intake of water and sodium was less favorable to growth but increased lignification of the tissues near the base of the stem. Pronounced cutinization occurred in the leaf epidermis of plants grown in distilled water, but plants from cultures in Knop's solution possessed fragile foliar epidermis. There was generally a rapid and strong lignification of all the tissues of plants grown in distilled water, but in Knop's solution this effect was not observed. Dassonville states that the growth of hemp and buckwheat was not influenced by the presence or absence of calcium or magnesium.

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[34] Hampe, W., Ueber Harnstoff und Harnsäure als stickstoffhaltige Pflanzennahrungsmittel. Landw. Versuchsst. **8**: 225-35. 1866.

[35] Nobbe, F., Ueber das relative Nahrungsbedürfniss der Pflanze. Landw. Versuchsst. **8**: 337-46. 1866.

[36] Nobbe, F., Beiträge zur Pflanzencultur in tropfbar flüssigen Wurzelmedien. VI. Ein Versuch zwei-jährige krautige Pflanzen in wässerigen Lösungen zu erziehen. Landw. Versuchsst. **9**: 228-34. 1867.

[37] Jodin, V., La rôle de la silice dans la végétation du mais. Ann. Chim. et Phys. V. **30**: 485-94. 1883.

[38] Kellner, O., Culturversuche mit Sumpfreis in wässerigen Lösungen. Landw. Versuchsst **30**: 23-32. 1884.

[39] Kreuzhage, C., and Wolf, E., Bedeutung der Kieselsäure für die Entwickelung der Haferpflanze. Landw. Versuchsst. **30**: 161-97. 1884.

[40] Dassonville, C., Influence des sels minéraux sur la forme et la structure des végétaux. Rev. Gen. Bot. **10**: 15-25, 59-68, 102-24, 161-70, 193-9, 238-60, 289-304, 335-44, 370-80. 1898.

Crone[41] proposed a nutrient solution which gave growth of barley, maize, rape and grape equal to that from parallel cultures in soil and which has later been considered especially satisfactory for cereal plants. In the course of an investigation of the influence of the phosphorus supply on several species of plants, Crone concluded that an excess of soluble phosphate (in solutions more concentrated than $\frac{n}{100}$ or $\frac{n}{50}$) induced chlorosis, even in the presence of a liberal supply of iron as ferrous sulphate. He also found that ferrous sulphate was toxic, while the corresponding phosphate was favorable to growth. Crone's solution contains, per liter, 1.0 g. KNO_3, 0.5 g. each of $CaSO_4$ and $MgSO_4$ and 0.25 g. each of $Fe_3(PO_4)_2$ and $Ca_3(PO_4)_2$. Some undissolved salts are present. From the results of his cultures he concluded that a satisfactory nutrient solution without undissolved salts was not possible.

In 1905 Harter[42] employed wheat and the method of water cultures to study the toxic effects of such salts as occur in alkali soils. Harter measured the rates of the elongation of roots in solutions of single salts. He concluded that each variety of a given species of plant must be tested separately to determine its toxic limits, for these limits are not necessarily the same in different varieties, a point deserving consideration in all nutrition experiments.

Studying the effect of the concentration of the nutrient solution on the growth of wheat and the influence of the addition of various salts to the solution, Breazeale[43] employed the water loss by transpiration as a measure of growth.[44] His fundamental nutrient solution contained $CaSO_4$, $MgHPO_4$, K_2CO_3, $NaNO_3$ and NH_4Cl, in chemically equivalent amounts, and its concentration ranged from 15 to 1550 parts per million (0.0015 to 0.1550 per cent.). From the growth of from 24 to 60 plants during periods of from 13 to 15 days, he concluded that concentration, independent of any changes in the chemical or nutrient properties of the solution, seemed to be important in determining plant growth. He placed the best concentration for wheat seedings at about 300 p.p.m. (0.03 per cent.).

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[41] Crone, G., Ergebnisse von Untersuchungen über die Wirkung der Phosphorsäure auf die höhere Pflanzen und eine neue Nährlösung. Sitzungsber. Niederrhein. Gesel. Nat.-und Heilkundee Bonn. **1902**: 167-73. 1902.

[42] Harter, L. L., The variability of wheat varieties in resistance to toxic salts. U. S. Dept. Agric. Bur. Plant Ind. Bull. 75. 1905.

[43] Breazeale, J. F., Effect of the concentration of the nutrient solution upon wheat cultures. Science., N. S. **22**: 146-9. 1905.

[44] See also: Livingston, B. E., Relation of transpiration to growth in wheat. Bot. Gaz. **40**: 178-95. 1905

At this period of the development of the method of water cultures Osterhout[45] called attention to the importance of physiologically balanced nutrient solutions. In his several experiments marine plants, as well as fresh water algae, liverworts, equisetum and several seed-plants were grown in diluted artificial sea water (*i.e.*, a nutrient solution of the chemical composition of diluted sea water) and in various combinations of NaCl, KCl, $MgCl_2$ and $CaCl_2$. The use of cuttings in place of seedlings was introduced for Tropaeolum and Tradescantia. Of the higher plants employed, wheat was grown 40 days in solutions having a concentration of $\frac{3m}{25}$, and the increase in total length of the root system was employed as a measure of growth. Osterhout concluded, in general, that the effect of increased concentration of any given nutrient solution, beyond a certain limit, leads to a toxic effect by some or all of the component salts, thus frequently necessitating a readjustment of the proportions of salts present in the solution or the further addition of new compounds. He found antagonisms to exist between sodium, on the one hand, and calcium and potassium on the other.

Hartwell, Wheeler and Pember[46] employed water cultures in experiments on the possibility of replacing potassium by sodium in nutrient media for plants. Wheat was used chiefly, but barley, rye, millet and oats were also employed, the plants being grown in bottles holding 250 cc. and the concentration of solutions ranging from 225 to 300 p.p.m., or from 0.0225 to 0.0300 per cent. The usual concentration used was 0.045 per cent. These authors' fundamental nutrient solution consisted of the following salts in the proportions given: 244 p.p.m. (0.0244 per cent.) $CaNO_3)_2$, 96 p.p.m. (0.0096 per cent.) $MgSO_4$, 58 p.p.m. (0.0058 per cent.) $CaH_4(PO_4)_2$ and a trace of $Fe_2(NO_3)_6$. To this solution were added equal parts by weight of the chloride, sulphate or carbonate of either potassium or sodium. The seeds were germinated by the method of the U. S. Bureau of Soils, as described by Livingston.[47] The solutions were usually replaced every three or four days. Growth was measured both by increase in green weight and by transpirational water loss, during periods of from 11 to 23 days, and determinations were made of the amounts of potassium removed from the various solutions.

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[45] Osterhout, W.J.V., On the importance of physiologically balanced solutions for plants. I. Marine plants. Bot. Gaz. **42**: 127-34. 1906.

——— On the importance of physiologically balanced solutions for plants. II. Fresh water and terrestrial plants. Bot. Gaz. **44**: 259-72. 1907.

[46] Hartwell, B.L., Wheeler, H.J., and Pember, F.R., The effect of the addition of sodium to deficient amounts of potassium, upon the growth of plants in both water and sand cultures. Ann. Report Rhode Island Agric. Exp. Sta. **20**: 299-357. 1907.

Hartwell, B.L. and Pember, F.R., Sodium as a partial substitute for potassium. Ann. Report Rhode Island Agric. Exp. Sta. **21**: 243-85. 1908.

The results of these studies indicated that sodium might, to some extent, replace potassium in the nutrient medium, but this possibility was less marked with rye than with the other plants employed. It was also observed that an increased amount of calcium was beneficial in nutrient solutions containing a large proportion of magnesium to calcium, the detrimental effect of an excess of the former element being more marked on transpiration and root development than on the development of tops. Some of the cultures here considered were conducted through periods of about three months (until the time of "shooting") in paraffin receptacles of 1500 cc. capacity. In these cultures the solutions were replaced weekly and ten or twenty plants were grown per jar, the production of dry matter in the crop being from ten to twenty times that in the other experiments. It was found that when the supply of potassium was deficient, but not sufficiently so to interfere with the apparent health of the plants, the addition of sodium to the nutrient solutions gave an increase in yield of from 19 to 30 per cent.

Hartwell and Pember[48] employed nutrient solutions for studying the effects of alkalinity and acidity of the medium on growth and for investigating the toxicity of ferrous sulphate. Wheat, rye, oats and barley were used, the three neutral solutions given below being employed as the basis of these cultures. Solution III was chiefly used. In all cases a small amount of a soluble ferric salt was added.

I. Knop's [65] solution (page 145), with K_2HPO_4 in place of KH_2PO_4.

II. As I, but with KNO_3 replaced by KCl and K_2HPO_4 by $CaH_4(PO_4)_2$.

III. As I, with 2/3 the usual amount of $Ca(NO_3)_2$, 11/8 the usual amount of KNO_3, twice the usual amount of $MgSO_4$, with NaH_2PO_4 in place of K_2HPO_4 and with NaCl added.

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[47] Livingston, B.E., A simple method for experiments with water cultures. Plant World **9**: 13-16. 1906.

[48] Hartwell, B.L., and Pember, F.R., The relation between the effects of liming, and of nutrient solutions containing different amounts of acid upon the growth of certain cereals. Ann. Report Rhode Island Agric. Exp. Sta. **20**: 358-80. 1907.

——— The relative toxicity of ferrous sulphate to barley and rye seedings. Ann. Report Rhode Island Agric. Exp. Sta. **21**: 286-94. 1908.

The concentrations of the salts in these solutions were as follows:

Salt	Solution I	Solution II	Solution III
	per cent.	*per cent.*	*per cent.*
$Ca(NO_3)_2$	0.0246	0.0246	0.0164
KNO_3	0.0081	......	0.0111
K_2HPO_4	0.0046	......	......
NaH_2PO_4	......	......	0.0032
$CaH_4(PO_4)_2$	......	0.0031	......
$MgSO_4$	0.0096	0.0096	0.0192
KCl	......	0.0060	......
NaCl	......	......	0.0049
Total Salts	0.0469	0.0433	0.0548

Hartwell and Pember state that " the nutrient solutions themselves were practically neutral, being slightly acid to phenolphthalein and slightly alkaline to methyl orange." To these solutions were added either various amounts of NaOH, various kinds and amounts of acids, or various amounts of $FeSO_4$. It was observed that in these cultures growth was not greatly influenced by any degree of alkalinity that did not cause precipitation. It was influenced but very little when the acidity was $\frac{n}{5000}$ or less. Moreover, the seedlings were found to render the nutrient solutions markedly alkaline after 23 days of growth. Barley and rye were equally retarded by $FeSO_4$, the latter salt seeming to be somewhat readily oxidized in the presence of the growing seedlings.

In the Bureau of Soils[49] of the U. S. Department of Agriculture the method of water cultures has been used very extensively. Beginning with the early work in which the nutrient values of soil extracts were compared, the Bureau has extended its investigations to include the influence upon growth of varying proportions of nutrient salts, the effect of organic constituents of the soil and the effect of the nutrient ions NO_3 K and PO_4 upon the poisonous properties of toxic compounds obtained from the soil.

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[49] Whitney, M., and Cameron, F.K., Investigations in soil fertility. U. S. Dept. Agric. Bur. Soils Bull. 23. 1904.

Livingston, B.E., Britton, J.C., and Reid, F.R., Studies on the properties of an unproductive soil. U. S. Dept. Agric. Bur. Soils Bull. 28. 1905.

Livingston, B.E., Further Studies on the properties of an unproductive soil. U. S. Dept. Agric. Bur. Soils Bull. 36. 1907.

Schreiner, O., and Reed, H.S., Certain organic constituents of soils in relation to soil fertility. U. S. Dept. Agric. Bur. Soils Bull. 47. 1907.

Schreiner, O., and Shorey, E.C., The isolation of harmful organic substances from soils. U. S. Dept. Agric. Bur. Soils Bull. 53. 1909.

Schreiner, O., and Reed, H. S., The role of oxidation in soil fertility. U. S. Dept. Agric. Bur. Soils Bull. 56. 1909.

Schreiner, O., and Skinner, J.J., Some effects of a harmful organic soil constituent. U. S. Dept. Agric. Bur. Soils Bull. 70. 1910.

Schreiner, O., and Skinner, J.J., Organic compounds and fertilizer action. U. S. Dept. Agric. Bur. Soils Bull. 77. 1911.

Skinner, J.J., A beneficial organic constituent of soils. Creatinine. U. S. Dept. Agric. Bur. Soils Bull. 83, Part III. 1911.

Schreiner, O., and Skinner, J.J., Nitrogenous soil constituents and their bearing on soil fertility. U. S. Dept. Agric. Bur Soils Bull. 87. 1912.

Schreiner and Skinner[50] have described an elaborate series of cultures with nutrient solutions, in which the three salts $NaNO_3$, K_2SO_4 and $CaH_4(PO_4)_2$ were employed, as supplying the nutrient elements in the compounds most common in commercial fertilizers. They expressed NO_3 in equivalent terms of NH_3, and expressed potassium as K_2O and phosphorus as P_2O_5. They employed a total concentration of 80 p.p.m. (0.008 per cent.) of the three components, K_2O, NH_3 and P_2O_5 together, and varied the partial concentration of each component in all possible ratios by increments of 10 per cent. Sodium, calcium and the ion SO_4 were not considered, although they were present in the solution and varied in amount. Each experiment consisted of a total of 66 cultures. Wheat, which has been used almost exclusively in this sort of work by the investigators of the U. S. Bureau of Soils, was germinated on perforated rubber or aluminum discs, which were floated on distilled water by means of corks or a raft of sealed glass tubing. When the shoots were about two centimeters long the seedlings were mounted in large-mouthed bottles holding 250 cc., ten plants to a bottle, in the manner described by Livingston [06, *1*]. The plants grew for 24 days, the nutrient solution being changed every three days and analysis being made of the residual solutions after each change. Special precaution was taken to render the distilled water fit for use in culture solutions by first treating it with thoroughly washed carbon black, in the way described by Livingston [07].

The results of this fundamental study were ingeniously plotted on triangular diagrams for purposes of comparison. It was found that growth was best in solutions which contained the respective equivalents of between ten and thirty per cent. P_2O_5, between thirty and sixty per cent. NH_3 and between thirty and sixty per cent. K_2O. There was thus indicated a rather wide range of adaptability of the plant in the absorption of nutrient elements from differently balanced media. In very early periods of growth the absorption of phosphorus was slow and of potassium rapid, while in the later periods more rapid absorption of NO_3 occurred. The greatest reduction in the concentration of the solutions occurred where growth was greatest, and solutions producing greatest growth suffered the least change in the original proportions of their salts. It thus appeared that a solution containing optimum proportions of the nutrient elements is possible. Where the amount of any one constituent in the solution was high, the plants absorbed an excess of this, which seemed not to be economically assimilated; to a certain extent, at least, concentration seems to influence absorption, as was observed with variant conclusions by Knop [61], Nobbe and Siegert [64, *1*], and Wolf [65]. In the later application of these culture solutions especially interesting results were obtained indicating the direct absorption of nitrogen in such organic compounds as creatinine and nucleic acid.

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[50] **Schreiner, O., and Skinner, J.J., Ratio of phosphate, nitrate and potassium on absorption and growth [*sic*]. Bot. Gaz. 50: 1-30. 1910. See also Schreiner and Skinner [10,1].**

Brown and MacIntire[51] and Brown and Beattie[52] have made use of water cultures in investigations of the quantities of nutrient elements removed from solution by several varieties of wheat. Germination was accomplished by the method of perforated plates as in the investigations of the U. S. Bureau of Soils previously mentioned. At intervals of four days, during total periods of from 12 or 16 days, the solutions were renewed, the residual solutions were restored to their original volumes and the amounts of NO_3, K and PO_4 were determined. The results as a whole were interpreted as indicating that some varieties possess greater absorbing power than do others.

True and Bartlett[53] investigated the concentration balance existing between pea seedlings and solutions of calcium and magnesium nitrates. These writers concluded that there is a definite concentration for each of these two salts, and for the mixture of the two, with which these roots absorb and excrete electrolytes at the same rate; that absorption surpasses excretion if the culture solution is initially more concentrated than at this equilibrium, and that excretion surpasses absorption if the solution is initially less concentrated. The molecular ratio of magnesium to calcium was found to determine the rate of absorption in these cultures, maximum absorption occurring with the ratio 1 : 1.

Pouget and Chouchak,[54] investigating Mitscherlich's "law of the minimum," employed a circulating culture solution, continuously renewed about the plant roots. Wheat seeds were germinated in distilled water and the seedlings were grown for three weeks in a nutrient solution containing the following salts in the proportions indicated: 0.025 per cent. K_2HPO_4, 0.025 per cent. KCl, 0.025 per cent. $MgSO_4$, 0.050 per cent. $CaSO_4$, 0.002 per cent. $FeCl_3$, and a variable amount of $Ca(NO_3)_2$. They found that the amount of NO_3 absorbed from the solution was proportional to the concentration of this radicle, up to a concentration from which the plant could no longer assimilate nitrate as fast as entrance occurred. The latter condition did not obtain for solutions containing 0.000165 per cent. of nitrogen (0.000965 per cent. of $Ca(NO_3)_2$). Above this concentration nitrate was absorbed in excess and accumulated in the plant, while at lower NO_3 concentrations, *i.e.*, with a deficient supply of nitrogen, the yield decreased with the concentration. They concluded that the absorption of any nutrient ion follows the "law of the minimum" and is proportional to the concentration of that ion in the medium, as long as it cannot be absorbed as rapidly as assimilation occurs.

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[51] Brown, B.E., and MacIntire, W.H., A comparative study of the removal of nutrient substances from culture solutions by different wheat varieties. Ann. Report Pennsylvania Agric. Exp. Sta. 1910-11. Pages 77-89. 1911.

[52] Brown, B.E., and Beattie, J.H., A further study on the removal of nutrient substances from culture solutions by different wheat varieties. Ann. Report Pennsylvania Agric. Exp. Sta. 1910-11. Pages 82-102. 1911.

[53] True, R.H., and Bartlett, H.H., Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. U. S. Dept. Agric. Bur. Plant Ind. Bull. 231. 1912.

[54] Pouget, I., and Chouchak, D., Influence de la concentration des solutions de substances nutritives sur leur absorption par les végétaux. Compt. Rend. Paris **154:** 1709-11. 1912.

——— Sur la loi du minimum. Compt. Rend. Paris **155:** 303-6. 1912.

In 1912 Breazeale and Le Clerc[55] published the results of a valuable study of the relation of plant growth in water cultures, to the chemical reaction of the culture medium. Wheat, clover and timothy were employed, the seeds being allowed to germinate and grow in pans, under the conditions employed for germination alone by the workers of the U. S. Bureau of Soils. Two successive crops of wheat were grown in distilled water, in solutions each containing 150 p.p.m (0.015 per cent.) of $NaNO_3$, of KCl or of K_2SO_4, and in solutions each containing 10 p.p.m. (0.001 per cent.) of HCl or of H_2SO_4. Duplicate cultures were conducted with and without the addition of 0.5 g. of $CaCO_3$ to each pan of about 3 liters of medium. The first crop grew nine and the second five days. The results were interpreted as indicating that the plant possesses ability to accommodate itself to a much greater degree of acidity in the medium when the acidity is gradually produced than when the solution is originally acid. It was shown that the seedlings grown in KCl or K_2SO_4 solution exerted a selective action, absorbing the potassium ion and leaving the Cl or SO_4 ion mainly in the external solution, which caused the latter to become acid and to retard root development. These writers considered such selective ionic absorption to be an important secondary cause of soil acidity.

Very recently Gile[56] has studied the ratio of lime to magnesia requisite for optimum plant growth, using water cultures. Rice was germinated on paraffined wire netting at the surface of distilled water and when the shoots were about 2.5 cm. long the seedlings were transferred to perforated paraffin stoppers in jars holding 550 cc., four plants being placed in each jar. The culture solutions, prepared from distilled water, were renewed every four days during the 40 or 50 days of growth, and at harvest the weights of oven dried tops and roots were obtained separately. Gile used a basal nutrient solution composed of the following salts in the concentrations given below.

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[55] Breazeale, J.F., and LeClerc, J.A., The growth of wheat seedlings as affected by acid or alkaline conditions. U. S. Dept. Agric. Bur. Chem. Bull. 149. 1912.

[56] Gile, P.L., Lime-magnesia ratio as influenced by concentration. Porto Rico Agric. Exp. Sta. Bull. 12. 1912.

Salt	Grams per 100,000 cc.	Grams per 100 cc.
KNO_3	10.71	0.01071
$NaNO_3$	21.43	0.02143
KH_2PO_4	7.14	0.00714
Na_2SO_4	3.15	0.00315
$FeSO_4$	1.61	0.00161
HCl	0.16	0.00016
Total Salt Content	44.20	0.04420

To this solution were added various amounts of $CaCl_2$ and $MgCl_2$. In solutions with a total concentration of from 0.1532 to 0.2162 per cent. toxic effects prevailed, but with total concentrations of from 0.0572 to 0.1062 per cent. no such effects were apparent.

With the concentration of calcium and of magnesium chloride ranging from 0.109 to 0.172 per cent., and with small amounts of the other salts present, the ratio 1 : 1, of lime to magnesia, was distinctly best. In similar solutions, where the calcium and magnesium chlorides were present in concentrations of from 0.023 to 0.062 per cent., the ratios of lime to magnesia between 10 : 1 and 1 : 10 were equally efficient. On the molecular basis $CaCl_2$ was found to be more toxic for rice than was $MgCl_2$. Gile concluded that the toxicity with which he dealt was related not simply to the ratio of lime to magnesia but to the relative concentrations of all of the salts. He also concluded that the proportions in which available lime and magnesia are present in ordinary soils do not affect the growth of plants, at least when the calcium and magnesium are present as chlorides.

SUMMARY OF PART I.

The preceding survey has shown that the history of the employment of water cultures may be divided into three rather distinct periods, the first ending about 1860, the second extending to about 1900, and the third extending from about the latter year to the present time.

In the first period the work of Woodward (about 1700) and of Duhamel du Monceau (about 1750) developed the conception of soil minerals as plant nutrients and paved the way for de Saussure's classic study of the assimilation of ash constituents, which appeared in 1804. The second period, including the extensive researches of Sachs, Knop, Nobbe and others, was very fruitful during the decade beginning about 1860. The work of these investigators placed the preparation of nutrient solutions and the conduct of water cultures upon a definite basis and permanently established the usefulness of this method for dealing with problems of mineral nutrient materials. Among the more important results obtained in this second period, mention may be made of the following:

(1) The optimum total salt concentration of the nutrient solution was found to be 0.3 per cent. or lower, if the solution were renewed frequently. (2) Differentiation of the essential from the non-essential nutrient elements was accomplished as far as this has been attained up to the present time. The essential elements were found to be nitrogen, sulphur, phosphorus, calcium, potassium, magnesium and iron, while chlorine and silicon were classed as probably only supplemental nutrient substances. (3) Standard formulae for nutrient solutions were proposed, that of Knop [65] being most generally recognized. (4) It was found that the composition of the nutrient solutions could vary widely without apparent detriment to the plant; hence it was considered that there was no single optimum nutrient solution. (5) Quantitative studies, especially those of Knop, indicated that the plant is able both to excrete and to absorb salts selectively, under different conditions. (6) It was generally believed that nutrient salts were absorbed by the plant without dissociation.

The third and present period of the investigation of water cultures has been characterized by a vigorously renewed activity, in which the method has been applied to current problems in plant nutrition. This activity has resulted in the development of new conceptions regarding the nature of the process of absorption and assimilation from nutrient solutions, and more accurate methods have been developed for following the process of absorption in a quantitative way. As a result of this work, it is now generally believed that the constituent ions rather than the complete salts are selectively absorbed by the plant, and that absorption by the latter thus influences both the composition and the chemical reaction of the nutrient solution. It has been shown also that the physiological balance of the nutrient elements in the solution, particularly that of calcium and magnesium, is important, and that this balance varies in a manner dependent both upon the composition and concentration of the solutions and upon the species of the plant. It appears also that the plant takes up an excess of nutrient elements beyond the requisite amount for maximum production, if such excess be present in the medium. Finally, some of the most recent work in this field has indicated that, within certain limits at least, a best or optimum proportion of the nutrient elements in the culture medium can be attained and that it is not absolutely essential for the latter to be absorbed in the form of inorganic compounds.

In concluding the present survey a comparison of the molecular composition of the more commonly employed nutrient solutions may be presented. Table I gives the formulae for these solutions (in terms of grams

TABLE I

Formulae of some commonly employed nutrient solutions at concentrations suitable for plant cultures

Author of Solution	Salts employed, grams per liter of solution												
	$Ca(NO_3)_2$	KNO_3	$NaNO_3$	KH_2PO_4	K_2HPO_4	$CaH_4(PO_4)_2$	$Ca_3(PO_4)_2$	$FePO_4$	$MgSO_4$	$CaSO_4$	K_2SO_4	KCl	$NaCl$
Crone [02]		1.00					0.25	0.25	0.25	0.25			
Detmer[57]	1.00				0.25				0.25			0.25	
Hartwell, Wheeler and Pember [07]	0.24					0.06			0.10			0.06	
Knop [65]	0.80	0.20		0.20					0.20				
Pfeffer[58]	0.80	0.20		0.20					0.20			0.10	
Sachs [60]		1.00					0.50		0.50	0.50			0.25
Schimper[59]	2.08	0.52			0.52				0.52				0.52
Schreiner and Skinner [10, *1* and *2*][60]			1.80			0.13					0.65		
Tollens [82]	1.00	0.25		0.25					0.24				0.15

[57] Detmer, W., Practical plant physiology, translated by S. A. Moor. London. 1898. Page 2.

[58] Pfeffer, W. [00], page 420.

[59] Schimper, A.F.W., Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora **73:** 207-61. Pages 220-1.

[60] An average of Schreiner and Skinner's best proportions of NH_3, K_2O and P_2O_5 is as follows: 36 p.p.m. each of NH_3 and K_2O, and 8 p.p.m. of P_2O_5. From these data have been calculated the corresponding number of grams per liter for the three salts actually present.

per liter) while table II shows the corresponding concentrations of the essential ions in molecular proportions. The notation of these tables is self-explanatory.

TABLE II

Volume-molecular compositions of the nutrient solutions given in table I

Author of Solution	Total salt concentration, *per cent.*	Volume-molecular concentration of essential ions[61]					
		Potassium	Calcium	Magnesium	Nitrate (NO_3)	Phosphate (PO_4)	Sulphate (SO_4)
Crone [02]........	0.200	.0050	.0022	.0010	.0050	.0017	.0020
Detmer [98].......	0.175	.0062	.0061	.0021	.0122	.0014	.0021
Hartwell, Wheeler and Pember [07].	0.046	.0008	.0017	.0008	.0030	.0005	.0008
Knop [65].........	0.140	.0035	.0049	.0017	.0117	.0015	.0017
Pfeffer [00]........	0.150	.0033	.0049	.0017	.0117	.0015	.0017
Sachs [60].........	0.275	.0100	.0098	.0045	.0099	.0032	.0079
Schimper [90].....	0.416	.0114	.0129	.0044	.0311	.0031	.0044
Schreiner and Skinner [10, *1* and *2*].	0.025	.0007	.0001	.0000	.0020	.0001	.0003
Tollens [82].......	0.190	.0043	.0060	.0020	.0146	.0018	.0020

The comparative data of table II show that great differences exist in the molecular concentrations and in the proportions of the nutrient ions as contained in these culture solutions. Excluding Schreiner and Skinner's incomplete solution, the ratio of potassium to magnesium is seen to vary from 1 : 1 to 5 : 1; that of calcium to magnesium, from 2 : 1 to 3 : 1; that of nitrate to phosphate, from 3 : 1 to 10 : 1; and that of nitrate to sulphate, from 1.3 : 1.0 to 7 : 1. These great discrepancies seem to justify a thorough investigation of the relative values of different proportions of the salts employed in such culture media, and the beginning of such a study is embodied in the work to be brought forward in the present paper. In this work Knop's solution was used as a basis. Part II deals with some of the properties of this solution.

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[61] In computing the data of table II, all the essential ions have been considered as existing in solution. The term volume-molecular is here and hereafter used to denote gram-molecules per liter.

PART II.

A CHEMICAL STUDY OF KNOP'S NUTRIENT SOLUTION.

THE SALTS.

The first step in preparing nutrient solutions on a quantitative basis is, obviously, to obtain salts of adequate purity, either dry or containing known amounts of water. In the present study " Baker analyzed " chemicals were used, including calcium nitrate in the form of fused lumps (labeled as containing 4 H_2O), di-potassium phosphate in the form of rather coarse granules, and potassium nitrate, mono-potassium phosphate, and magnesium sulphate in crystalline form, the last containing the usual seven molecules of water.

Literature was consulted and tests were made to determine at what temperatures these salts could be dried to constant weight. Thorpe[62] states that on evaporating a solution of calcium nitrate the salt is recovered in the anhydrous state; and that hydrated magnesium sulphate loses six molecules of water at 160° C. and the seventh molecule at 200° C. According to Watts,[63] calcium nitrate containing four molecules of water boils at 132° C. after dissolving in its water of crystallization, and remains clear until about one-third of this water is lost, when the anhydrous salt deposits. The same writer states that di-potassium phosphate is converted to potassium pyrophosphate ($2\ K_2HPO_4 = H_2O + K_4P_2O_7$) on strongly heating, the action being non-reversible by boiling water. Tests made upon calcium nitrate in the present work showed that this salt can be dried to the anhydrous state at 150° C. After drying a mass of this salt at this temperature for about fifteen hours, pulverizing it and dividing into lots of from 25 to 50 g., four to six hours further drying at 150° is required. On drying hydrated magnesium sulphate in the same manner, but at 155° to 160° C., it lost weight equivalent to six molecules of water and could be treated as containing one molecule of water. At 150° C. di-potassium phosphate dried to constant weight and remained soluble in water, but at about 180° C. it lost 4.76 per cent. of weight and became only very slightly soluble even in dilute acids. The change undoubtedly involved the formation of pyrophosphate, which, if complete, would cause a loss of 5.17 per cent. of the original weight.[64] Mono-potassium phosphate and potassium nitrate were found stable at temperatures up to 150° C.

It was necessary to dry all the calcium nitrate used in this work, in the manner described, on account of the varying amounts of water in the lumps of salt. Some lots of this salt, moreover, contained approximately two instead of four molecules of water. In the earlier stages of the work magnesium sulphate was also dried, account being taken of the molecule of water remaining, but the stock supply of this salt used in the later work contained no free moisture and was therefore weighed without drying. The other salts were dried at 105° C. in the analytical work, but in the greater part of the culture work to be described subsequently they were weighed directly and allowance was made for the water present as determined from samples, which was considerable only in the case of di-potassium phosphate. Moisture determinations on small samples of the salts were made in cylindrical weighing bottles with ground glass stoppers, while the large quantities of salts were dried in pairs of watch glasses, 10 cm. in diameter, each pair held together by means of a brass clip while cooling in the desiccator. The preliminary drying of calcium nitrate and magnesium sulphate was accomplished in crystallizing dishes 10 cm. in diameter. All weighings in this work were made to a tenth of a milligram.

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[62] Thorpe, T.E., Dictionary of applied chemistry **1:** 401. London. 1890; **2:** 494. London. 1891.

[63] Watts, H., Dictionary of applied chemistry **3:** 511. London. 1892; **4:** 113. London. 1894.

[64] This salt should be tested before using; it sometimes contains pyrophosphate.

PREPARATION OF THE STOCK SOLUTIONS.

Knop's solution, as commonly used at the present time, contains four parts of calcium nitrate and one part each of potassium nitrate, magnesium sulphate, and either mono- or di-potassium phosphate, by weight. Plainly, the chief consideration in preparing this solution should be given to avoiding the addition of calcium nitrate to either magnesium sulphate or potassium phosphate in a concentrated solution, as such procedure would cause the precipitation of either calcium sulphate or calcium phosphate. Tollens[65] has described a stock nutrient solution, similar to Knop's and free from precipitate, which is prepared in three parts. Magnesium sulphate and mono-potassium phosphate are each dissolved alone and the required volume of each solution is added to as large a volume of water as is allowed by the required final concentration. To the resulting solution is then added the third stock solution containing potassium and calcium nitrates. Knop[66] himself has described a stock solution following his formula, which he devised to avoid the precipitation of di-calcium phosphate. He dissolved magnesium sulphate alone to form one of the two primary stock solutions and added phosphoric acid to the final complete solution in order to prevent precipitation. Livingston[67] has called attention to the importance, in preparing Knop's solution, of keeping calcium nitrate and di-potassium phosphate separate until the final dilutions are made, in order to avoid the formations of di-calcium phosphate as noted by Klebs[68] and others.

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[65] Tollens, B., Ueber einige Erleichterungen bei der Cultur von Pflanzen in wässerigen Lösungen. Jour. f. Landw. **30**: 537-40. 1882.

[66] Knop, W., Bereitung einer concentrierten Nährstofflösungen für Pflanzen. Landw. Versuchsst. **30**: 292-4. 1882.

[67] Livingston, B.E., On the nature of the stimulus which causes the change of form in polymorphic green algae. Bot. Gaz. **30**: 289-317. 1900. Page 294.

Preliminary tests related to the question of precipitates in this solution showed that equal parts by weight of magnesium sulphate and either mono- or di-potassium phosphate could be mixed in considerable concentration, without precipitation. In the case of di-potassium phosphate the limit of stable concentration in a solution of the two salts (in equal amounts by weight) was equivalent to 9.0 per cent. of the complete part B, and no precipitate formed after a period of 14 days; although weaker solutions accumulated a slight, but negligible, flocculent sediment after several days, apparently as a result of action of the solution upon the glass of the containing vessel. With mono-potassium phosphate and magnesium sulphate the limit of stability of a solution containing equal amounts by weight was equivalent to 10.0 per cent. of the complete part B after a period of 15 days. These tests were made at temperatures of from 20° to 23° C. and the results just given cannot be expected to apply to higher temperatures, which would doubtless increase the velocities of the reactions causing precipitation. Addition of potassium nitrate to these solutions, in the proportion required by Knop's formula, did not alter their stability.

Following the results of the preceding tests, and in order to have the separate stock solutions of nearly equal concentration, Knop's solution was prepared for the present work in two parts, part A containing calcium nitrate, and part B containing the other three salts. The highest possible concentrations of these two solutions, as established by the tests just described, is 12.0 per cent. for part A and 9.0 per cent. for part B, when di-potassium phosphate is employed and 12.33 per cent. and 10.0 per cent., respectively, when mono-potassium phosphate is used. The concentrations employed in this study were 8.0 per cent. for part A and 6.0 per cent. for part B, which provided a wide margin of safety against precipitation in part B. The latter solution therefore contained 2 per cent. of each of its constituent salts. Equal volumes of these two stock solutions unite to form a 7.0 per cent. Knop's solution; but the lower concentrations usually required are to be obtained by adding the necessary volumes of the stock solutions to a large volume of water in the volumetric flask.

CONCENTRATION LIMITS OF STABLE SOLUTIONS.

By the preceding method 100 cc. samples of Knop's solution were prepared over a wide range of concentrations, to determine the stability of this medium at room temperatures. These temperatures ranged from 20° to 22° C. in the earlier stages of the test, but later rose to 26° C. The solution containing di-potassium phosphate showed a slight precipitate after four days in a 2.4 per cent. concentration, but not until after 40 days did precipitate appear in a 2.0 per cent. solution, and the precipitate in the latter case remained slight even after 76 days. When mono-potassium phosphate was employed a precipitate formed after four days in a 3.0 per cent. solution, but not until after 40 days in a 2.0 per cent. solution, this precipitate remaining slight after 145 days. It is apparent, therefore, that complete Knop's solution of 2.0 per cent. strength and containing either of the potassium phosphates may be used as a stock solution. For cultures where the solutions are to be renewed every three or four days, it may be considered safe, excluding the influence of the growing plants, to employ 2.2 or 2.8 per cent. concentration, according as di- or mono-potassium phosphate is used. Of course, the influence of the plants in promoting precipitation is likely to be a serious factor, but these concentrations are far above the optima for growth, at least for wheat seedlings, as will be shown in Part III of the present paper.

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[68] Klebs, G., Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena. 1896. Page 8

NATURE OF CHANGES CAUSING PRECIPITATION IN KNOP'S SOLUTION.

Attention was given to determining the nature and extent of the changes which cause the precipitation here dealt with, and attempts to recover the salts remaining in solution developed unforeseen difficulties. The solutions with precipitate were thoroughly shaken and filtered through paper, without previous wetting of the filter and without washing the residue. Duplicate small quantities of the filtrate (5 or 10 cc., depending upon the original concentration) were then evaporated in weighed porcelain dishes on an asbestos plate over a low flame. The residues from evaporation were dried four hours at 160° C., cooled in a desiccator and weighed.

Precipitation when KH_2PO_4 is employed.

A trial of this procedure on duplicate portions (5.0 cc.) of a freshly prepared 7.0 per cent. Knop's solution containing KH_2PO_4 showed a loss of 17.4 per cent. of the total salts originally present, substitution of a water-bath for the asbestos board reduced this loss to 7.86 per cent. It appeared, therefore, that some volatile substance, presumably an acid radicle such as that of nitric acid, must have been liberated from the solutions during the process of concentration. The following equations and data will serve to illustrate the probable manner in which this loss occurred.

$$Ca(NO_3)_2 + MgSO_4 \rightleftarrows CaSO_4 + Mg(NO_3)_2$$

$$3\,Ca(NO_3)_2 + 2\,KH_2PO_4 \rightleftarrows Ca_3(PO_4)_2 + 2\,KNO_3 + 4\,HNO_3$$

By the calculation of the chemical equivalents for 100 cc. of the 7.0 per cent. solution the following data were derived from the above equations.

(1)	Ca converted to $CaSO_4$	0.3331 g.	
	Ca converted to $Ca_3(PO_4)_2$	0.4419 g.	
	Total Ca converted	0.7750 g.	
(2)	Total Ca in original solution	0.9776 g.	
	Total Ca converted	0.7750 g.	
	Ca unconverted	0.2026 g.	
(3)	Total NO_3 of $Ca(NO_3)_2$ in original solution	3.0224 g.	
	NO_3 equivalent to unconverted Ca	0.6264 g.	
	NO_3 liberated from $Ca(NO_3)_2$	2.3960 g.	
(4)	Total Mg in original solution, 0.2018 g., equivalent to	1.0300 g.	NO_3
	Total K in KH_2PO_4 in original solution, 0.2837 g. equivalent to	0.4555 g.	NO_3
	NO_3 retained by Mg and K	1.4855 g.	
(5)	NO_3 liberated from $Ca(NO_3)_2$	2.3960 g.	
	NO_3 retained by Mg and K	1.4855 g.	
	NO_3 completely liberated	0.9105 g.	

The last quantity, 0.9105 g. NO_3, corresponds to 0.9253 g. HNO_3, which would be 13.22 per cent. of the original salt content of the solution. This calculated loss is much higher than that (7.86 per cent.) which actually occurred by evaporating on the water bath. It seems possible either that some of the HNO_3 considered in this calculation as liberated may have been retained, by adsorption on the solid residue or otherwise, during evaporation and drying, or that the reactions indicated may have been incomplete. Whatever may have been the cause of this retention of NO_3 it seems remarkable that the amounts of residue recovered from duplicate portions of the solution agreed within one milligram, on weights of about 300 milligrams, by both methods of recovery. It seems surprising that the amount of retention, if due to purely physical causes, should agree so closely in the duplicate solutions, without special precautions to maintain uniform conditions during the process of recovery.

To determine why the calculated loss was exceeded by that occurring when evaporation was conducted on the asbestos plate, the several salts of Knop's solution were evaporated in this manner from their separate solutions. It was found that magnesium nitrate lost from 16.0 to 18.0 per cent. of its weight under these conditions. On approaching dryness a simple solution of this salt showed an acid reaction in litmus paper suspended several centimeters above its surface. It also showed the test for nitrate on a porcelain surface moistened by diphenylamine reagent and similarly suspended, indicating that dissociation of this salt and a consequent loss of nitric acid probably played an important part in the production of the high loss first observed in recovering the salts from the 7.0 per cent. Knop's solution.

Means were sought for retaining the nitric acid apparently lost on the water bath, but it was impracticable to proceed far in this direction. Zinc dust was found to be more effective than sodium carbonate, but zinc nitrate dissociates to some extent at 160° C., the temperature required for drying the recovered salts. The addition of from 0.2 to 0.3 g. of zinc dust to 100 cc. of the 7.0 per cent. Knop's solution, before proceeding with evaporation, reduced the loss of salts to 3.29 per cent., which further reduces to 3.08 per cent. if account is taken of 0.21 per cent. of hydrogen necessarily lost when the free nitric acid combines with the zinc. The percentages of salts recovered by the above method agreed well for duplicate portions of the solutions, but since the original proportions of the salts were altered by the precipitation occurring in some solutions, it is clear that no general correcting factor can be derived to apply to all these cases. As a means of approximating the amounts of salts actually present in the solutions however, the salts recovered have been increased by 3.08 per cent., which is the amount just stated to be unaccounted for after the use of zinc dust. In this manner the data of table III were obtained.

TABLE III

Loss of total salts from solution in Knop's medium employing KH_2PO_4, where precipitation occurred.

	Original concentration of solution, *per cent.*	Salts precipitated *per cent.* of total.	Actual final concentration of solution, *per cent.*
Series 1, after standing 62 days	3.00	3.74	2.89
	3.50	13.36	3.03
	4.00	14.60	3.42
	4.50	16.10	3.78
	5.00	17.40	4.13
Series 2, after standing 145 days	3.00	11.50	2.65
	4.00	11.70	3.53
	5.00	14.30	4.28

The data of table III show that, soon after the limit of stability is passed, the decrease of concentration due to precipitation becomes very pronounced. They do not indicate, however, the nature of the changes which occur in the process of precipitation. Further study was devoted to this question. Microscopic examination showed that the precipitate consisted chiefly of the characteristic spherical aggregates of monoclinic crystals of $CaSO_4 \cdot 2H_2O$, although $Ca_3(PO_4)_2$ appeared also to be present to a slight extent. Gravimetric determinations were therefore made to determine the losses of calcium, phosphorus and sulphur from solution. The standard methods which were followed may be briefly indicated here.

Calcium was precipitated from duplicate samples (10 cc.) of the final solution, as oxalate, in the presence of acetic acid, and converted to $CaSO_4$ for determination. Phosphorus was determined in the filtrate from the calcium determination, employing double precipitation by magnesia mixture in the presence of NH_4Cl, and igniting both precipitates to give the pyrophosphate. Sulphur was determined from separate samples (5 cc.) of the final solution, by precipitating with $BaCl_2$, in dilute HCl solution. Series 1 of table III gave the results presented in table IV.

TABLE IV

Losses of calcium, phosphorus and sulphur from Knop's solution employing KH_2PO_4, after standing 62 days, in terms of the total amounts of these elements originally present.

Original total concentration of the solution, *per cent.*	Loss of calcium, *per cent.* of the original calcium content	Loss of phosphorus, *per cent.* of the original phosphorus content	Loss of sulphur, *per cent.* of the original sulphur content
3.00	2.86	11.22	3.50
3.50	23.93	12.28	65.40
4.00	25.58	6.61	71.10
4.50	27.23	6.80	74.30
5.00	28.65	7.36	74.70

The data of table IV assume significance when the losses of the acidic elements, sulphur and phosphorus, are compared with the loss of the basic element calcium; and also, when the combined losses of these three elements are compared with the actual losses of total salts as previously reported. Assuming that all of the sulphur and phosphorus lost from solution formed $CaSO_4$ and $Ca_3(PO_4)_2$ respectively, the amount of calcium which should thus be removed from solution may be calculated and expressed as percentage of the total amount originally present. The latter percentage is to be compared with the percentage actually removed. These considerations are presented in table V.

In the weakest solution considered in table V, where the total loss of salts was comparatively small, the combined loss of phosphate and sulphur, expressed in terms of an equivalent amount of calcium, considerably exceeds the actually observed loss of calcium. The fact that this excess falls chiefly upon the phosphorus, together with the well-known susceptibility of soluble phosphates to adsorptive attraction, suggests that part of the actual loss of phosphorus here observed may be ascribed to adsorption of some of the

TABLE V

Comparison of the amount of calcium chemically equivalent to the losses of sulphate and phosphate with the amount of calcium actually lost from Knop's solution employing KH_2PO_4.

Original total concentration of solution, *per cent.*	*Per cent.* of total calcium equivalent to actual loss of			Actual loss of calcium *per cent.* of total calcium
	SO_4	PO_4	SO_4+PO_4	
3.00	1.20	5.30	6.50	2.86
3.50	22.53	5.81	28.34	23.93
4.00	24.45	3.12	27.57	25.58
4.50	25.45	3.21	28.66	27.23
5.00	25.58	3.48	29.06	28.65

mono-potassium phosphate on the precipitate.[69] The calculated and actual losses of calcium agree much better in the remaining cases, approaching each other closely with the higher concentrations. It is shown by these data that the precipitate of the higher concentrations of Knop's solution consists chiefly of $CaSO_4$ with small amounts of calcium phosphate, probably $Ca_3(PO_4)_2$, as was indicated, indeed, by the previous microscopic examination.

Calculating the preceding losses as percentages of the total original amounts of Ca, SO_4 and PO_4, they may be compared with the actual losses so expressed, as previously given in table III. Such a comparison is presented in table VI.

TABLE VI

Comparison of the calculated losses from Knop's solution (employing KH_2PO_4) of Ca, SO_4 and PO_4 and the calculated loss of Ca, SO_4 and PO_4 together, with the actually observed total loss.

Concentration of original solution, *per cent.*	Calculated loss from solution (*per cent.* of total salts) of				Actual loss *per cent.* of total salts
	Ca	SO_4	PO_4	$Ca+SO_4+PO_4$	
3.00	0.40	0.40	1.12	1.92	3.74
3.50	3.34	7.48	1.25	12.05	13.36
4.00	3.56	8.13	0.66	12.35	14.60
4.50	3.80	8.50	0.68	12.98	16.10
5.00	3.99	8.54	0.74	13.27	17.40

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[69] The relative solubility of the precipitate itself precluded washing it free from either absorbed or occluded salts. Even had such procedure been possible, it would have lessened the value of the subsequent analyses by destroying the balance upon which these unstable solutions would have supplied salts to plants growing therein.

The data of table VI show that not all the salts removed from solution are accounted for by precipitation of $CaSO_4$ and $Ca_3(PO_4)_2$, for the actual loss is, in all cases, greater than the calculated. Moreover, the excess of the actual over the calculated value increases as the amount of precipitate increases. This condition finds rational explanation in the supposition that soluble salts may be adsorbed by the precipitate and thus removed from solution. This latter phenomenon should probably be considered as increasing the disturbance of the original balance of salts, already disturbed by the precipitation itself.

Precipitation When K_2HPO_4 is Employed.

The tests just described were applied also to Knop's solution prepared with K_2HPO_4, instead of KH_2PO_4, as the source of phosphorus. When evaporated on a water bath, this solution (with a concentration of 7.0 per cent.) showed a loss of 9.46 per cent. of the total salts originally present. By the addition of about 0.3 g. zinc dust to 5.0 cc. of the solution before evaporating on the bath, the loss was reduced to 3.69 per cent.

Calculating, for 100 cc. of the 7.0 per cent. solution, the chemical equivalents which would be involved in the rearrangements probably occurring during evaporation, the following equations and data are derived, which correspond to those for the other solution given on pages 163-4.

$$Ca(NO_3)_2 + MgSO_4 \rightleftarrows CaSO_4 + Mg(NO_3)_2$$

$$3\ Ca(NO_3)_2 + 2\ K_2HPO_4 \rightleftarrows Ca_3(PO_4)_2 + 4\ KNO_3 + 2\ HNO_3$$

(1)	Ca converted to $CaSO_4$	0.3331 g.
	Ca converted to $Ca_3(PO_4)_2$	0.3453 g.
	Total Ca converted	0.6784 g.
(2)	Total Ca in original solution	0.9776 g.
	Total Ca converted	0.6784 g.
	Ca unconverted	0.2992 g.
(3)	Total NO_3 of $Ca(NO_3)_2$ in original solution	3.0224 g.
	NO_3 equivalent to unconverted Ca	0.9251 g.
	NO_3 liberated from $Ca(NO_3)_2$	2.0973 g.
(4)	Total Mg of orig. solution, 0.2018 g., equivalent to	1.0300 g. NO_3
	Total K of original K_2HPO_4, 0.4490 g., equivalent to	0.7119 g. NO_3
	NO_3 retained by Mg and K	1,7419 g.
(5)	NO_3 liberated from $Ca(NO_3)_2$	2.0973 g.
	NO_3 retained by Mg and K	1.7419 g.
	NO_3 completely liberated	0.3554 g.

The last quantity, 0.3554 g. NO_3, corresponds to 0.3611 g. HNO_3, which would be 5.16 per cent. of the original salt content of the solution. This result differs from the case (page 164) where KH_2PO_4 was employed in the solution, in that the calculated loss is much *lower* (while in the other case it was much higher) than that which actually occurred (9.46 per cent.) by evaporation on the water-bath. If the amount of hydrogen necessarily lost by the reaction between HNO_3 and zinc (which is 0.08 per cent. of the total salts) be deducted, the actual loss on evaporation where zinc was present becomes 3.61 per cent. The last value is about 20 per cent. greater than the corresponding value (3.08 per cent., page 165) obtained for the solution where KH_2PO_4 was used instead of K_2HPO_4.

The nature of the disturbance of balance was also investigated in the solutions with K_2HPO_4 where precipitation occurred, with the results presented in table VII.

TABLE VII

Loss of total salts from solution in Knop's medium employing K_2HPO_4, where precipitation occurred, after standing 76 days.

Original concentration of solution, *per cent.*	Salts precipitated, *per cent.* of total	Actual final concentration of solution *per cent.*
2.00	2.12	1.96
2.20	4.78	2.09
2.40	9.47	2.17
2.60	8.85	2.37
2.80	11.20	2.49
3.00	12.60	2.62

These data show that the loss of salts from solution increases as the concentration of the latter increases, and that the final strengths of the solutions fall increasingly short of the original strength. The deficiency for the solution of 3 per cent. strength is considerably greater than that which occurred when KH_2PO_4 was present, for a nearly equal period of standing (table III). Data were obtained relative to the nature of the changes which cause precipitation in some of these cases. Calcium and phosphate were determined in duplicate samples (15 cc.) and sulphur in duplicate samples (10 cc.) of the filtered solutions, following the methods previously applied where KH_2PO_4 was used in the original solution. The results of this study are given in table VIII.

The most striking feature indicated by the data of table VIII is the absence of phosphorus in the precipitate, in which this solution differs markedly from that made with KH_2PO_4. Assuming the decrease of sulphur to be due to the formation of $CaSO_4$, the relations shown in table IX are brought out.

TABLE VIII

Losses of calcium, phosphorus and sulphur from Knop's solution employing K_2HPO_4, after standing 76 days, in terms of the total amounts of these elements originally present.

Original total concentration of the solution, *per cent.*	Loss of calcium, *per cent.* of original calcium content	Loss of phosphorus, *per cent.* of original phosphorus content	Loss of sulphur, *per cent.* of original sulphur content
2.00	5.01	0.00	13.2
2.40	17.33	0.00	46.2
3.00	21.18	0.00	57.0

Table IX shows clearly that the precipitate here consists almost entirely of $CaSO_4$, as in the solutions containing KH_2PO_4; but the non-removal of phosphorus from solution is here in marked contrast with the result obtained with those solutions.

TABLE IX

Comparison of the amounts of calcium chemically equivalent to the losses of sulphate and phosphate, with the amount of calcium actually lost from Knop's solution employing K_2HPO_4.

Original total concentration of solution, *per cent.*	*Per cent.* of total calcium equivalent to actual loss of SO_4	Actual loss of calcium, *per cent.* of total calcium
2.00	4.52	5.01
2.40	15.83	17.33
3.00	19.53	21.18

The data of table X show the comparison between the sum of the calculated losses of calcium and sulphate and the actual loss of total salts from these solutions employing K_2HPO_4.

TABLE X

Comparison of the calculated losses from Knop's solution (employing K_2HPO_4), of Ca and SO_4, and the calculated loss of Ca and SO_4 together, with the actually observed total loss.

Concentration of original solution, *per cent.*	Calculated loss from solution (*per cent.* of total salts) of			Actual loss, *per cent.* of total salts
	Ca	SO_4	Ca + SO_4	
2.00	0.70	1.51	2.21	2.12
2.40	2.41	5.28	7.69	9.47
3.00	2.95	6.52	9.47	12.60

The data given in table X show that, while the calculated loss of $CaSO_4$ and the actual total loss of soluble salts agree well in instances where slight precipitation occurred, the actual loss increasingly exceeds the calculated loss as the amount of precipitate increases. This, as in the cases already considered (where KH_2PO_4 was employed in the original solution page 168), suggests that the precipitate probably removes soluble salts from solution by adsorption. It seems reasonable to believe that this phenomenon will modify the supplying of salts to the plants in such disturbed solutions, just as it does in the soil, rendering their availability to the organism subject to the absorbing power of the latter and the solvent power of the surrounding, residual solution. Contrary to the previously quoted idea (Knop [82], Klebs [96], Livingston [00]) that the insoluble compound formed in these solutions is $CaHPO_4$, the present results show conclusively that the formation of $CaSO_4$ is the chief obstacle to the preparation of concentrated Knop's solution.

SUMMARY OF PART II.

In the preceding paragraphs methods have been described by which may be obtained the desired weights of the salts employed in preparing Knop's nutrient solution for plant cultures.

It has been found convenient to employ two parts in preparing concentrated stock solutions according to Knop's formula, one part (A) containing only calcium nitrate and the other part (B) containing the remaining three salts. The limit of stability of part B, which determines the maximum possible strength of such stock solutions, was found to be 10.0 per cent. when KH_2PO_4 was employed as the source of phosphorus, and 9.0 per cent. when K_2HPO_4 was used. The concentrations of part A are limited to 12.33 per cent. and 12.00 per cent. for solutions prepared with KH_2PO_4 and with K_2HPO_4, respectively. These limits apply to solutions standing for a period of from 14 to 15 days at temperatures of from 20° to 23° C. It was further found that, exclusive of the influence of the growing plants, the complete Knop's nutrient solution is stable for from three to four days at temperatures of from 20° to 26° C., at a concentration of 2.2 per cent., when K_2HPO_4 is employed, and at a concentration of 2.8 per cent. when KH_2PO_4 furnishes the phosphorus. From the point of view of stability of the solutions these results establish a preference for KH_2PO_4 over K_2HPO_4 in the preparation of this solution.

Analytical investigation of the changes occurring in Knop's solution at concentrations above the limit of stability, showed that precipitation was due almost entirely to the formation of $CaSO_4$. Considerable emphasis upon this point seems justified in view of the apparently general belief among physiologists, supported by statements in the literature of nutrient solutions, that precipitation is due to the formation of calcium phosphate.

This investigation also showed that the change of concentration of the nutrient solution becomes serious not far above its limit of stability, and that, as a result of this precipitation and the adsorptive effects which apparently attend it, the composition of the solution, and probably also its nutritive value, is greatly altered. These conditions must be taken into account in conducting culture work with strong solutions.

Incidentally, the interesting fact was observed that nitric acid is liberated by the rearrangement of acids and bases which occurs in evaporating Knop's solution to dryness, so that quantitative recovery of the total salts by this method is impossible, unless certain modifications are introduced into the usual procedure, such as the addition of some basic element before proceeding with the evaporation.

PART III.

EXPERIMENTS WITH YOUNG WHEAT PLANTS IN WATER CULTURES.

INTRODUCTION.

The nutrition experiments now to be described dealt with the growth of young wheat plants in three series of nutrient solutions. These series differed widely in total concentration, and therefore in osmotic pressure, but the different members of each series had approximately the same osmotic pressure. Their concentrations were based upon the results of preliminary cultures in Knop's solution and were so chosen that one series was favorable, a second was too weak and the third was too strong for optimum growth. By varying the proportion of salts in Knop's formula but maintaining approximately constant osmotic pressure, in a manner to be subsequently described, the best proportions of these salts for the early stages of growth of wheat were determined for the three different concentrations or osmotic pressures used. The results of these cultures, which covered approximately the first third of the full growth period of wheat, may not, of course, be assumed to be applicable to the later stages of growth, especially to the period including seed development, in which the demand for nutrient salts probably undergoes considerable alteration. The determination of the best proportions of salts for these later stages may be considered, therefore, as distinct problems for future investigation.

METHODS.

The vessels used in the preliminary cultures were pint glass jars of the "Mason" type, conveniently holding 400 cc. of solution. Because of the necessity for frequent changes of solution it was found later more economical of salts to replace these jars, for the greater part of the work, by colorless flint glass bottles of 250 cc. capacity. These bottles were 7 cm. in diameter outside at the base and 10.2 cm. high, with a shoulder 7.8 cm. above the base and a neck 1.5 cm. high and 4.5 cm. in diameter inside at the opening. Both the jars and bottles were covered with heavy black paper when in use.

As in other water culture work with such plants, it was necessary to support the seedlings so as to allow their roots to project downward into the solution in the jar, while the seeds were kept well above the surface. The method of supporting seedlings in wedge shaped openings in the margin of a flat cork stopper, described by Livingston [06] and used in the water culture work of the U. S. Bureau of Soils (Livingston [07], Schreiner and Skinner [10, *1* and *2*], etc.), while very satisfactory in general, has certain disadvantages as far as convenience of operation is concerned, and some of these disadvantages are overcome by a method devised in the present work. Flat corks 1.6 cm. thick and 5.7 cm. in smaller diameter were found well suited for use with the "Mason" jars. These were first perforated in the center by the removal of a slightly conical core having a smaller diameter of 3 cm. This was accomplished by rotating the cork upon the projecting spindle of a Westinghouse "general utility" electric motor, a second cork previously placed upon the spindle serving as a backing against which the first cork rested. A sharp, thin knife blade, held against a suitable support which also acted as a gauge, was then slowly advanced into the rotating cork, in such manner as to cut out a slightly tapering core with its conical surface parallel to the edge of the cork stopper itself.[70] Six equidistant perforations 0.7 cm. in diameter were then made, with a cork borer, in the cork ring thus formed, these perforations slightly overlapping the inner margin of the ring so as to open laterally into the central space from which the core had been removed. The seedlings were wrapped just above the still attached seed in enough cotton to wedge them into position and were slipped laterally from the large central opening into the six holes just mentioned. After the six plants were in place, the central core was inserted and pressed firmly into position. This method gave very good support to the seedlings. When the growing plants required further support, as when the leaves began to droop, a cylindrical wooden staff, 30 cm. long and 0.6 cm. in diameter, tapered at its lower end, was tightly inserted in the opening previously formed by the motor spindle at the center of the cork core. A somewhat rigid upright support was thus provided at the center of each group of plants, to which they might be secured

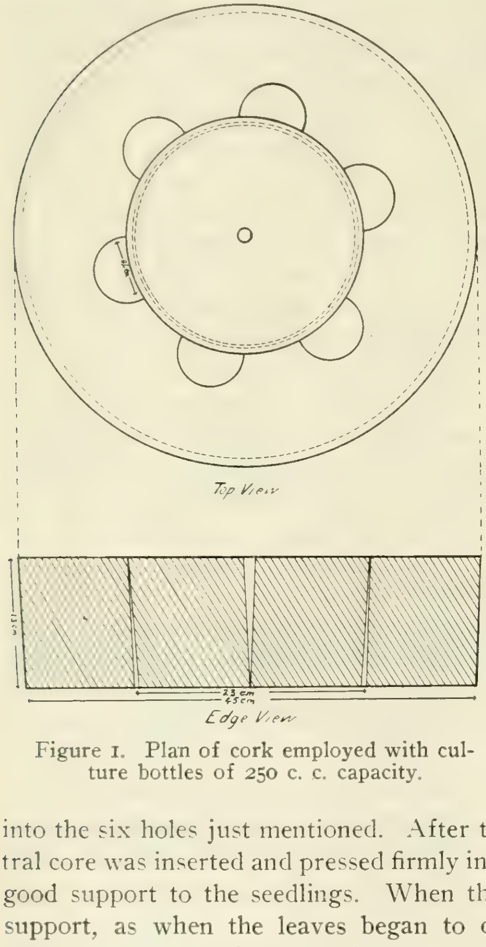

Figure 1. Plan of cork employed with culture bottles of 250 c. c. capacity.

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[70] This method of cutting the corks was suggested to the writer by Dr. Lon A. Hawkins, now of the U. S. Dept. of Agriculture, Bureau of Plant Industry.

by tying with light cord. The form of the completed cork is shown in figure 1.

The flat corks selected for the bottles that replaced the jars in later work were 1.3 cm. thick and 4.5 cm. in smaller diameter. A core 2.3 cm. in smaller diameter was removed by the method above described and six perforations 0.6 cm. in diameter were cut on the inner margin of the cork ring so formed. Before using the corks thus prepared they were thoroughly coated by immersion in melted paraffin. This treatment expanded them somewhat and frequently rendered them too large for good service, so that it was necessary slightly to reduce them in size, by rotating them on the motor spindle against a coarse file. A cork 4.2 cm. in smaller diameter is to be recommended in place of the size above mentioned, if the same bottles be employed.

The solutions used in this work were prepared from the same salts and in the same manner as that described in Part II (page 160). Distilled water was obtained from a "Barnstead" still, with tin-lined condenser. It was stored in a large stoneware jar until drawn (through a block tin tube) into a glass carboy for immediate use. Stock solutions were stored in glass bottles previously cleaned by thorough treatment with the usual cleaning mixture of potassium bichromate in sulphuric acid. In addition to the four chief salts, iron was supplied to the earlier cultures by adding to each jar, at each change of solution, five drops of an approximately 5 per cent. solution of $FeSO_4$. The stock solution of this salt soon hydrolyzed, however, forming a precipitate of ferric hydroxide and acquiring a strong acid reaction, and this was replaced in succeeding culture series by a suspension of ferric phosphate. The latter was prepared by precipitating ferric chloride solution with mono-sodium phosphate and ammonium hydroxide, washing the precipitate free from chlorine, dissolving it in dilute nitric acid, reprecipitating by ammonium hydroxide and washing the final precipitate free from the nitrate radicle. The resulting gelatinous precipitate was placed in such volume of water as, when thoroughly shaken, gave a uniform suspension containing 0.0024 g. of ferric phosphate per cubic centimeter. Two drops of this suspension were added to each culture vessel whenever the solution was changed, so that each culture received, in the course of the eight changes of solution made in each series, 0.0019 g. of ferric phosphate or 0.0007 g. of iron. A concentration of 0.0 per cent., *i.e.,* distilled water, was employed as a control culture in each series.

The wheat used in this work was a vigorous stock of the Fulcaster variety which was supplied by the U. S. Department of Agriculture, Bureau of Plant Industry. It gave a test of 92.0 per cent. total, and 73.0 per cent. strong germination. Very satisfactory uniformity in size and vigor of the seedlings was obtained by germinating the seeds after the following method, which is a modification of that employed by Livingston [06] and other workers (Schreiner and Skinner [10, *1* and *2*], etc.) in the U. S. Bureau of Soils. A single thickness of common mosquito netting was thoroughly coated by immersion in melted paraffin, rendered uniformly porous by crumpling, and firmly stretched over the top of a circular "granite-ware" enamelled cake pan, being held in place by heavy twine drawn very tight by means of a wooden twister. This cake pan, which was 21.9 cm. in bottom diameter and 3.1 cm. deep, with nearly vertical sides, was placed in a similar but deeper pan, 25.6 cm. in diameter at the bottom, and sufficient distilled water was added to just cover the mosquito netting. After a preliminary soaking in distilled water for about three hours the seeds were uniformly distributed upon the stretched netting and the water level was maintained thereafter by means of an inverted bottle of water serving as a Mariotte flask. By changing the water daily at first and more frequently when excreted material began to accumulate rapidly, satisfactory uniform seedlings about 5 cm. high were obtained after five days in the experiment greenhouse. The plantlets were then placed in the culture vessels in the manner already described.

The well known and important influence of the aerial environment upon growth seems to justify a somewhat detailed account of the conditions under which the cultures were grown in the present work. Both germination and growth were conducted in one of the experiment greenhouses, in the early part of the work without shade, later with a cheese cloth curtain and finally, as the spring season advanced, with the usual white lead coating (of medium density) upon the glass. Another greenhouse intervened between the one employed and the vestibule connecting with the laboratory rooms, and the door between the two greenhouses was kept closed excepting when in use. The ventilators of the intervening greenhouse as well as that actually in use were open as constantly as the condition of the weather permitted. Furthermore, a ventilating flue through which a current of air is always passing outward is arranged to withdraw contaminated air from the vestibule, which is guarded by two swinging doors. It thus appears that the cultures here considered were absolutely free from any possible effects of gases from the laboratories. The outside air could not have been greatly contaminated, for the Johns Hopkins Laboratory of Plant Physiology is situated in the extreme outskirts of Baltimore, in the midst of extensive park and forested country.

The experiment greenhouse was somewhat drier than is usual in greenhouses, having a cement floor and containing but relatively few plants. During the period covered by the chief series of experiments the evaporating power of the air in the greenhouse ranged from 3.48 to 48.18 cc.

per day, from the Livingston standard porous cup atmometer,[71] and the temperature ranged approximately from 20° to 43° C. Only once, however, was this upper extreme of temperature even closely approached.

In all cases the plants grew 24 days after being placed in the nutrient solution and the latter were changed on the seventh, fourteenth and twenty-first days of growth, where "Mason" jars were used, and every third day in the remaining series. During the progress of the cultures relative development of tops and of roots and any unusual phenomena of growth were recorded. At the end of the 24-day period the plants were severed just above the remains of the seed, washed free from salts and the roots and tops dried separately at a temperature of from 50° to 60° C., for two or three days. Finally, the separate parts of the plants were dried at about 102° C., employing the 4-inch watch glasses mentioned in Part II (page 161). Two hours of drying at this temperature were found sufficient to bring them to constant weight. Excepting in the last series, where the practice appeared to be unjustified on account of the adverse effect of high concentration of the nutrient solutions, the few cultures where a single plant was missing from a culture (because of death or elimination on account of obviously inherent inferiority) have been brought into uniformity with the others by proportionately increasing the total dry weight actually obtained, so that it represents six plants.

TREATMENT OF THE NUTRIENT SOLUTION IN TERMS OF OSMOTIC PRESSURE.

Throughout the following discussion concentrations will be expressed in terms of percentage of total dissolved salts (grams per 100 cc. of solution) and also in atmospheres of osmotic pressure. Since the latter treatment is unusual, the method by which the osmotic pressures have been derived will be given.

These pressures have been calculated on the basis of the data of electrolytic dissociation given by Jones [12], for all the salts employed except mono-potassium phosphate. Data for the latter salt are not found in Jones' tables. They were determined for use in the present work by Dr. E. P. Wightman[72] of Professor

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[71] Livingston, B.E., A simple atmometer. Science, N. S. **28:** 319-20. 1908.

———, Operation of the porous cup atmometer. Plant World, **13:** 111-8. 1910.

———, A rotating table for standardizing porous cup atmometers. Plant World **15:** 157-62. 1912.

[72] The following percentages of dissociation at 25 degrees C. were obtained for monopotassium phosphate. The notation is the same as that of Jones [12].

Concentration of Solution	V	U	Dissociation
Per cent.			*Per cent.*
0.140	97.25	90.61	78.8
0.086	158.32	91.79	79.8
0.014	972.50	99.25	86.3
—	—	115.00 (assumed)	100.0

Jones' laboratory. All data were taken at 25° C., which approximated the average temperature to which the culture solutions were subjected in the greenhouse. The foregoing procedure is based upon the well known fact that osmotic pressure is a colligative or additive property, *i.e.*, it depends upon the total number of particles (ions, molecules and molecular aggregates) in solution, irrespective of their kind.[73] After first deriving the percentage concentration of each salt in any given concentration of Knop's solution, the equivalent of this in molecular concentration was obtained by dividing the percentage value by the percentage value of a molecular solution of the salt in question. Then, by interpolation in Jones' [12] tables, was derived the corresponding percentage of dissociation. Let us suppose that in this manner a dissociation value of 50 per cent. has been obtained for a molecular solution of potassium nitrate, which dissociates into two ions, K and NO_3. In this case an average of 0.5 gram-molecule of the salt remains undissociated while the remaining 0.5 gram-molecule has produced two ions and become osmotically equal to an undissociated molecular solution. Hence, the total osmotic value of the solution under consideration is 1.5 times the molecular value. This value of 1.5, representing the number of osmotically active parts per molecule, resulting from dissociation, will be termed the *osmotic factor.* The value obtained from multiplying this osmotic factor by the corresponding molecular concentration, which has been taken as 1.0 in this illustration, will be termed the *osmotic concentration.* The latter is 1.5 in this illustration. An approximation to the total osmotic concentration of any solution may be obtained by summing the values for the partial concentrations of the constituent salts, as illustrated by the data of table XI, for a 1.0 per cent. Knop's solution.

TABLE XI

Partial concentrations of the four component salts in Knop's solution with total concentration of 1.0 per cent.

Salt	Osmotic factor	Concentration			
		Per cent.	Volume-molecular		
			Decimal fraction	Common fraction	Osmotic
K_2HPO_4	1.93	0.1429	0.0093	*m*/107.5	0.0180
$MgSO_4$	1.58	0.1429	0.0119	*m*/ 84.1	0.0188
$Ca(NO_3)_2$	2.52	0.5714	0.0349	*m*/ 28.7	0.0880
KNO_3	1.89	0.1429	0.0142	*m*/ 70.5	0.0270
Total	....	1.0	......	......	0.1518

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[73] Nernst, W., Theoretical chemistry, translated by H. T. Tizard. London. 1911. Page 258.

While the calculation of the osmotic factor is relatively simple for salts of monobasic acids, and can be determined readily in such cases by arithmetical treatment, its determination becomes a more complicated problem where several different ions may be successively formed during the process of diluting the solution, as in the case of salts of trivalent acids like phosphoric acid. The following scheme illustrates the complexity of electrolytic dissociation in a solution of mono-potassium phosphate, where the process is incomplete and proceeds by stages with increasing dilution.

Stage I. KH_2PO_4;
Stage II. (1) KH_2PO_4, (2) K, (3) H_2PO_4;
Stage III. (1) KH_2PO_4, (2) K, (3) H_2PO_4, (4) H, (5) HPO_4;
Stage IV. (1) KH_2PO_4, (2) K, (3) H_2PO_4, (4) H, (5) HPO_4, (6) H, (7) PO_4.

It will thus be seen that seven separate particles, six of which are chemically different, may result from incomplete dissociation of a single molecule of this salt, each of these numbers being reduced by one when dissociation is complete. In such cases calculation may be simplified by algebraical treatment summing the progressive dissociation step by step. Thus if M is chosen to represent the original number of molecules of KH_2PO_4, and x, y and z represent the percentages of dissociation of KH_2PO_4, H_2PO_4 and HPO_4, respectively, a general formula (which is applicable to any salt) may be derived. The following equations bring this out, the numbers in brackets corresponding to those in the scheme of dissociation just given.

[I] $M\ KH_2PO_4$ (x per cent. dissociation)=

[II, 1] $\left(M-\frac{Mx}{100}\right) KH_2PO_4+$

[II, 2] $\frac{Mx}{100} K +$

[II, 3] $\frac{Mx}{100} H_2PO_4$. Total number of particles at this stage $= M + \frac{Mx}{100}$.

[II, 3] $\frac{Mx}{100} H_2PO_4$ (y per cent. dissociation)=

[III, 3] $\left(\frac{Mx}{100}-\frac{Mxy}{10{,}000}\right) H_2PO_4 +$

[III, 4] $\frac{Mxy}{10{,}000} H +$

[III, 5] $\frac{Mxy}{10{,}000} HPO_4$. Total number of particles at this stage $= M + \frac{Mx}{100} + \frac{Mxy}{10{,}000}$

[III, 5] $\frac{Mxy}{10{,}000} HPO_4$ (z per cent. dissociation)=

[IV, 5] $\left(\frac{Mxy}{10{,}000}-\frac{Mxyz}{1{,}000{,}000}\right) HPO_4 +$

[IV, 6] $\frac{Mxyz}{1,000,000}$ H +

[IV, 7] $\frac{Mxyz}{1,000,000}$ PO_4. Total number of particles at this stage =

$$M + \frac{Mx}{100} + \frac{Mxy}{10,000} + \frac{Mxyz}{1,000,000}.$$

In the preceding general formula the value of M is the molecular concentration of the solution to be considered. The value of x is determined from conductivity data. The values of y and z used for the phosphates of potassium in the present work have been based upon statements of Stieglitz[74] and of Lehfeldt.[75] According to the latter writer, the electrolytic dissociation of H_2PO_4 is about equal to that of acetic acid and that of HPO_4 is comparable to that of an alcohol, which is quite negligible.[76] On the basis of these statements Jones' [12] values for molecularly equivalent concentrations of acetic acid have been taken for the dissociation of H_2PO_4, and the probably slight dissociation of HPO_4 has been neglected. Dissociation of magnesium sulphate and of calcium nitrate have been considered as occurring in one step in each case, thus:

$$MgSO_4 \rightleftarrows Mg + SO_4$$
$$Ca(NO_3)_2 \rightleftarrows Ca + 2\ NO_3$$

This treatment of the former salt seems to be supported by Nernst ([11], page 510), but the latter salt probably dissociates in two steps. The present state of our knowledge of this subject, however, appears to justify no more detailed treatment than has been given here.

It should be stated that the determination of dissociation effects in a complex mixture of salts is not so simple a matter as the preceding treatment indicates. In such mixed solutions the influence of one salt upon another, in modifying the extent of dissociation, needs consideration. While Nernst states that dissociation equilibrium under these conditions can be determined accurately by calculation, he adds that this is attended by considerable difficulty (Nernst [11], page 517); and it is clearly apparent that the complex of factors influencing dissociation in Knop's solution is not as yet readily amenable to wholly satisfactory mathematical treatment. The neglect of the inter-influence of salts in the present work is therefore unavoidable, as far as the calculations are concerned. The method here employed, however, seems to provide the nearest approach to accurate determination of osmotic pressure, with the exception of measurement of the lowering of the freezing point, which was impracticable at the time when this work was done.[77]

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[74] Stieglitz, J., The elements of qualitative chemical analysis. 1: 102. New York. 1912.

[75] Lehfeldt, H., Electrochemistry. 1: 104. London. 1912.

[76] Good agreement with the latter statement is shown by the insignificance of the last fraction in the general formula just derived.

Conductivity measurements were kindly made by Dr. E. P. Wightman upon samples of the nutrient solutions used for water cultures in the subsequent part of this work. These are given in table XII; they are of interest because of the partial light that they throw upon the relative dissociation effects of the several pairs of salts and of the more complex mixtures in Knop's solution. For comparison, the conductivities of the several solutions have been calculated from Jones' [12] data, assuming no influence of one salt upon another.

TABLE XII

Observed and calculated specific electric conductivities of simple and complex salt solutions as they occur in a Knop's medium of 0.6 per cent. concentration.

Solution	Component salts	Concentration, *per cent.*	Specific conductivity		
			Observed (a)	Calculated (b)	Difference between observed and calculated (b-a)
Part A, complete.	$Ca(NO_3)_2$	0.343	0.4069	0.4107	0.0038+
Partial mixture (1).	$Ca(NO_3)_2$	0.343	0.4721	0.5078	0.0157+
	$MgSO_4$	0.086			
Partial mixture (2).	$Ca(NO_3)_2$	0.343	0.4536	0.4688	0.0152+
	KH_2PO_4	0.086			
Partial mixture (3).	$MgSO_4$	0.086	0.1561	0.1590	0.0029+
	KH_2PO_4	0.086			
Part B, complete	$MgSO_4$	0.086	0.2820	0.2635	0.0185−
	KH_2PO_4	0.086			
	KNO_3	0.086			
Complete Knop's solution.	$Ca(NO_3)_2$	0.343	0.6101	0.6742	0.0641+
	$MgSO_4$	0.086			
	KH_2PO_4	0.086			
	KNO_3	0.086			

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[77] For a discussion of the nature and measurement of osmotic pressure, see Nernst [11], Book I, Chap. 5. As the present paper goes to press, Mr. J. W. Shive, of the Laboratory of Plant Physiology of the Johns Hopkins University, is completing the freezing-point determinations of the entire series of nutrient solutions here dealt with. His results, showing the variations between the present author's approximate calculations of osmotic pressure and the corresponding values derived by cryoscopy, will appear in the Plant World, 1914.

Because of the possible rearrangements between salts, which may or may not seriously alter the number of ions in solution, the drawing of definite conclusions from the above data would be clearly unjustifiable. There seems to be some significance, however, in the fact that the solution of magnesium sulphate and potassium phosphate, which has been shown in Part II (page 162) to be quite stable, shows close agreement of the measured with the calculated conductivity; while the solution of calcium nitrate and magnesium sulphate, which the results of Part II (page 167) indicate as characterized by extensive rearrangement, shows the greatest discrepancy between these two conductivity values, with the exception of the complete Knop's solution. Apparently the chief disturbance of conductivity and of osmotic pressure, as effected by the inter-influence of salts, should be attributed to the reaction between calcium nitrate and magnesium sulphate, which becomes evident in higher concentrations by precipitation of calcium sulphate. It will be observed that the depression of the actual below the calculated conductivity is about 10 per cent. for the complete Knop's solution.

Calculation of the osmotic pressure from the values for osmotic concentration has been based upon the most accurate direct measurements of osmotic pressure extant, namely the data of Morse and his coworkers[78] for cane sugar solutions, interpolation being made in their tables for a temperature of 25° C. The solutions in question were first converted[79] to the weight-normal basis employed by Morse *et al.*, to gram-molecules of solute per 1000 g. of solvent, employing Brix's specific gravity tables for sucrose solutions with $d = \left(\frac{17.5°}{17.5°}\right)$ (Landolt and Börnstein [94]).

PRELIMINARY CULTURES IN KNOP'S SOLUTION.

Following the general methods already described, cultures were grown in nutrient solutions prepared according to Knop's formula, both with di- and with mono-potassium phosphate as the source of phosphorus, to determine the relative efficiencies of these two phosphates and the optimum concentrations of the two nutrient solutions thus prepared. Series 1 and 2 ranged in concentration, by increments of 0.2 per cent., from 0.0 to 2.0 per cent. (0.0 to 8.15 atmospheres), and established the general range of optimum concentrations. Series 3 and 4 ranged in concentration, by increments of 0.05 per cent., from 0.0 to 0.8 per cent. (0.0 to 3.3 atmospheres), including also a solution of 0.01 per cent. concentration. The latter series extended over the general optimum range established by the preceding series. Equal volumes of the two stock nutrient solutions (part A with a concentration of 8.0 per cent. and part B of 6.0 per cent.) were drawn from burettes, with mixing, into as large a volume of water in a volumetric flask as the required solution would permit, thus avoiding the immediate formation of precipitate in the more concentrated solutions. The volume of the flask used was 800 cc. in series 1 and 2, thus providing for duplicate cultures in each one of the several concentrations. In series 3 and 4 the flask employed had a capacity of 250 cc.

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[78] Morse, H.N., Holland, W.W., Zies, E.G., Myers, C.N., Clark, W.M., and Gill, E.E., The relation of osmotic pressure to temperature. Part V. The measurements. Amer. Chem. Jour. **45:** 554-603. 1911. Page 602.

[79] Landoldt, H., and Börnstein, R., Physikalische-chemische Tabellen. Berlin. 1894. Page 231.

Series 1 (Knop's solution, various concentrations, employing di-potassium phosphate).

These culture's grew during the 24 days from December 7, 1912, to January 1, 1913, in Knop's solution with di-potassium phosphate. Culture jars each having a capacity of 400 cc. were employed and the solutions were renewed on the seventh, fourteenth and twenty-first days of growth.

All of the solutions in this series formed flocculent precipitates after the third day of growth, and during the periods between subsequent renewals of solution. Retardation of the development of secondary roots was observed in the solutions having a concentration of 1.4 per cent. (5.40 atmospheres osmotic pressure) and above, while secondary roots were almost completely suppressed in 2.0 per cent. solution (7.95 atmospheres osmotic pressure). After nine days the plants in 1.8 and 2.0 per cent. concentrations (7.15 and 7.95 atmospheres osmotic pressure) were apparently dying. After 20 days of growth, the tops appeared nearly alike in concentrations of from 0.2 to 1.2 per cent. (0.85 to 4.75 atmospheres pressure). After nine days, and also after 24 days, the residual solutions were found to be acid to litmus paper, with the exception of the controls in distilled water, which remained neutral. Death phenomena became conspicuous in the control plants after 22 days. The yields of this series, obtained as already described, will be presented with those of series 2.

Series 2 (Knop's solution, various concentrations, employing mono-potassium phosphate).

Cultures of this series grew for the time period of 24 days, from December 22, 1912 to January 16, 1913. It was conducted in the same manner as was series 1, excepting that phosphorus was here supplied in the form of mono-potassium phosphate instead of the di-potassium salt. When the cultures were set up the seedlings appeared to be somewhat smaller than were those of series 1 at its beginning, although they were of the same age.

Only at and above a concentration of 1.0 per cent. (4.05 atmospheres of osmotic pressure) did any precipitate form in this series during the seven days elapsing between renewals of the solutions. On the eighth day of the entire period some retardation in growth of secondary roots was apparent in the solution with 1.0 per cent. concentration (4.05 atmospheres of pressure), this effect being marked in the concentration of 1.2 per cent. (4.85 atmospheres), and extreme in and above that of 1.6 per cent. (6.45 atmospheres). It was also noted on the eighth day that optimum growth of tops appeared to extend from the solution with 0.2 per cent. concentration (0.85 atmospheres) to that with a concentration of 0.8 per cent. (3.30 atmospheres). After 21 days retardation of root development remained as before, but the tops were quite uniform in appearance up to the concentration of 1.6 per cent. (6.45 atmospheres), from which point growth declined noticeably toward the upper limit of the series.

The dry weights of tops and roots of the plants from the various cultures of series 1 and series 2 are presented in tables XIII and XIV.

TABLE XIII

Dry weights of tops of duplicate cultures of wheat, six plants per culture, grown 24 days in various concentrations of Knop's solution with K_2HPO_4 (series 1) and with KH_2PO_4 (series 2).

Concentration of medium	Approximate osmotic pressure of medium		Dry weight of tops from duplicate cultures, A and B					
			Series 1, with K_2HPO_4			Series 2, with KH_2PO_4		
	Series 1, with K_2HPO_4	Series 2, with KH_2PO_4	Actual weight		Average weight, relative to control as unity	Actual weight		Average weight, relative to control as unity
			Culture A	Culture B		Culture A	Culture B	
Per cent.	*Atmospheres*	*Atmospheres*	*Grams*	*Grams*		*Grams*	*Grams*	
0.0	0.00	0.00	0.1116*	0.0982	1.00	0.1102	0.1472	1.00
0.2	0.85	0.85	0.5476	0.5530	5.27	0.3924	0.5770	3.77
0.4	1.65	1.70	0.4696	0.4336	4.30	0.4406	0.3434	3.05
0.6	2.45	2.50	0.5790*	0.5130	5.20	0.4408	0.5814	3.96
0.8	3.25	3.30	0.5244	0.4716	4.75	0.4578	0.4396	3.49
1.0	3.95	4.05	0.4220	0.4686*	4.25	0.3864	0.3396	2.82
1.2	4.75	4.85	0.3198	0.4908*	4.34	0.3500	0.4214	3.00
1.4	5.40	5.65	0.3594*	0.4002	3.62	0.3284	0.3568	2.67
1.6	6.30	6.45	0.3747	0.3384	3.40	0.3042	0.3108*	2.39
1.8	7.15	7.30	0.1506*	0.3256	2.27	0.2218	0.2592	1.87
2.0	7.95	8.15	0.1042	0.1518	1.22	0.2334*	0.2454	1.86

***Calculated from weight of five plants.**

The relative weights are plotted as graphs in figure 2, in which abscissae represent the approximate osmotic pressures of the medium and ordinates are proportional to the relative dry weights. Since the weights from the control grown in distilled water are always taken as unity the graphs all pass through the same point (0.1) and are therefore directly comparable, as far as the method of plotting is concerned. Since, however, the two

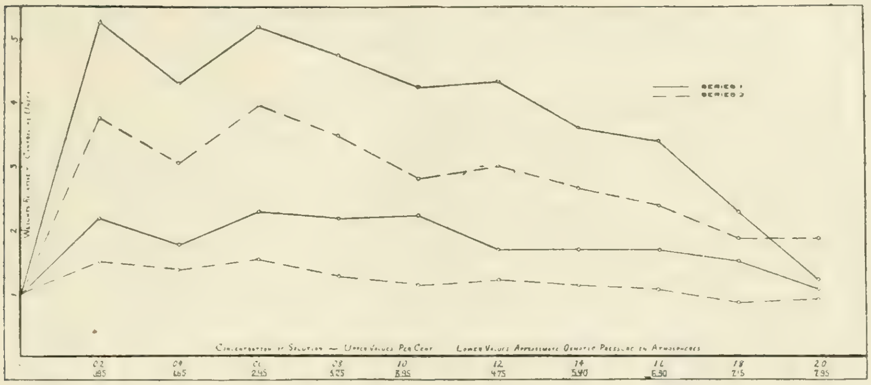

Figure 2. Graphs of dry weights of wheat plants, series 1 and 2. Upper graphs, tops; lower graphs, roots.

series were not carried out simultaneously, it is clear that direct quantitative comparisons between them are not possible; the aerial conditions were of course not precisely the same during the two time periods, which may account for differences between the average absolute dry weight obtained

TABLE XIV

Dry weights of roots of duplicate cultures of wheat, six plants per culture, grown 24 days in various concentrations of Knop's solution with K_2HPO_4 (series 1) and with KH_2PO_4 (series 2).

Concentration of medium	Approximate osmotic pressure of medium		Dry weight of roots from duplicate cultures, A and B					
			Series 1, with K_2HPO_4			Series 2, with KH_2PO_4		
	Series 1, with K_2HPO_4	Series 2, with KH_2PO_4	Actual weight		Average weight, relative to control as unity	Actual weight		Average weight, relative to control as unity
			Culture A	Culture B		Culture A	Culture B	
Per cent.	*Atmospheres*	*Atmospheres*	*Grams*	*Grams*		*Grams*	*Grams*	
0.0	0.00	0.00	0.1326*	0.1020	1.00	0.1080	0.1148	1.00
0.2	0.85	0.85	0.1884	0.2252	2.19	0.1430	0.1938	1.51
0.4	1.65	1.70	0.2242	0.1914	1.77	0.1638	0.1425	1.37
0.6	2.45	2.50	0.2922*	0.2462	2.29	0.1468	0.1974	1.54
0.8	3.25	3.30	0.2768	0.2372	2.19	0.1540	0.1289	1.27
1.0	3.95	4.05	0.1768	0.2358*	2.24	0.1326	0.1218	1.14
1.2	4.75	4.85	0.1567	0.2400*	1.69	0.1170	0.1543	1.22
1.4	5.40	5.65	0.1812*	0.2154	1.69	0.1244	0.1306	1.14
1.6	6.30	6.45	0.2074	0.1866	1.68	0.1194	0.1200*	1.07
1.8	7.15	7.30	0.1740*	0.1810	1.51	0.0954	0.0930	0.85
2.0	7.95	8.15	0.1170	0.1344*	1.07	0.1056*	0.0958	0.90

*Calculated from weight of five plants.

from the distilled water controls in the two series (see tables XIII and XIV). The graphs show good agreement in the relations of tops to roots and the general trends of variation in tops and roots are similar in the two series, the latter agreement being especially true of the tops.

For both series the greatest dry weights were from solutions with approximate osmotic pressures ranging from 0.85, or less, to 4.85 atmospheres (0.2 to 1.2 per cent. concentration). The greatest weight of tops is shown for the solution with an approximate osmotic pressure of from 2.45 to 2.50 atmospheres (0.6 per cent. concentration). With the latter concentration the relative yield of tops where mono-potassium phosphate was used (series 2) was only 76.15 per cent. of that obtained where the di-potassium salt was employed; but, as has been remarked previously, having been grown partly at different time periods the two series are not to be considered as directly comparable. Furthermore, on account of the precipitation which occurred in all the solutions of series 1 and in the higher concentrations of series 2, it is hardly to be assumed that the two series are strictly comparable on the basis of their chemical compositions as made up.

Series 3 (Knop's solution, various concentrations, employing di-potassium phosphate).

This and the following series were planned to determine the optimum concentration of Knop's solution more closely than was accomplished by series 1 and 2. The concentrations employed ranged from 0.0 per cent. (distilled water control) to 0.8 per cent. (0.00 to 3.30 atmospheres of osmotic pressure) by increments of 0.05 per cent., excepting that a concentration of 0.01 per cent. (0.05 atmospheres of pressure) was introduced. The culture vessels were the large-mouthed bottles previously described (page 173) and the cultures were grown singly instead of in duplicate, the solutions being renewed once in three days. Precipitated ferric phosphate was used in these series as source of iron, being added to the solutions in the manner already described (page 175), and the phosphorus was supplied as either di- or mono-potassium phosphate.

Series 3 extended over the 24-day period from January 12 to February 6, 1913, and employed di-potassium phosphate. The solutions were renewed every three days. At the first renewal all of them contained a slight amount of precipitate. This was apparently due to rather high temperatures caused by direct sunshine falling at this time upon the culture bottles and the slate table top upon which the latter stood. A cheesecloth shade was introduced after these three days, to prevent the access of direct sunshine to the cultures, and no further formation of precipitate was observed. No marked differences in the growth of plants were apparent among the several solutions, although all the solution cultures exhibited a much more pronounced growth than did the control in distilled water. The data of yields will be presented with those of series 4.

Series 4 (Knop's solution, various concentrations, employing mono-potassium phosphate).

This series grew during the 24 days from January 24 to February 17, 1913, and was like series 3 in all particulars excepting that mono-potassium phosphate was used instead of the di-potassium salt. Since the first half of the period of this series was identical with the last half of the period of series 3, the aerial conditions influencing growth in the two series were probably more nearly the same than in the case of series 1 and 2, and the yields are therefore more directly comparable here than in the former pair of series.

A slight flocculent precipitate, nearly white or reddish in color, was

TABLE XV

Dry weights of tops and roots of cultures of wheat, six plants per culture, grown 24 days in various concentrations of Knop's solution with K_2HPO_4 (series 3) and with KH_2PO_4 (series 4).

Concentration of medium	Approximate osmotic pressure of medium		Dry weight, relative to control as unity			
	Series 3 (with K_2HPO_4)	Series 4 (with KH_2PO_4)	Tops		Roots	
			Series 3	Series 4	Series 3	Series 4
Per cent.	*Atmospheres*	*Atmospheres*				
0.00	0.00	0.00	1.00 (.1128*)	1.00 (.1294)	1.00 (.0954*)	1.00 (.0984)
0.01	0.05	0.05	3.73	3.67	1.39	2.28
0.05	0.25	0.25	3.31	5.89	1.15	1.83
0.10	0.45	0.45	4.63	6.01	1.34	1.92
0.15	0.65	0.65	4.24	5.65	1.36	1.78
0.20	0.85	0.85	4.58	5.53	1.38	2.14
0.25	1.05	1.10	3.88	5.88	1.28	1.85
0.30	1.25	1.30	4.17	5.64	1.26	1.86
0.35	1.45	1.50	3.91	6.02	1.39	1.89
0.40	1.65	1.70	3.98	5.10	1.13	1.64
0.45	1.85	1.90	4.48	5.27	1.49	1.81
0.50	2.05	2.10	4.13*	4.88	1.47*	1.72
0.55	2.25	2.30	5.11*	5.43	1.94*	1.75
0.60	2.45	2.50	4.67	5.26	1.57	1.75
0.65	2.65	2.70	3.98	5.81	1.32	1.98
0.70	2.85	2.90	3.43	5.73	1.21	1.89
0.75	3.05	3.10	4.72	5.93	1.63	2.04
0.80	3.25	3.30	4.47	5.89	1.85	1.90

*Calculated from weight of five plants.

apparent at each renewal of the solutions of series 4, after each three-day interval. This precipitate appeared to be related to the presence of the insoluble ferric phosphate rather than to any serious disturbance of equilibrium among the dissolved salts. All of the solution cultures produced excellent growth, much superior to that of the control, but no marked differences among the solution cultures themselves were detected at any time during the period.

The dry weights of the plants from series 3 and 4 are presented together in table XV in which the arrangement of the items is similar to that employed in tables XIII and XIV. In table XV, however, the actual weights have been suppressed, to save space, excepting in the cases of the controls in distilled water, where these actual weights are given (in grams) in parenthesis beneath the relative weight 1.00; the actual weight in any other case may be readily obtained by multiplying the corresponding relative weight by the actual weight of the proper control culture.

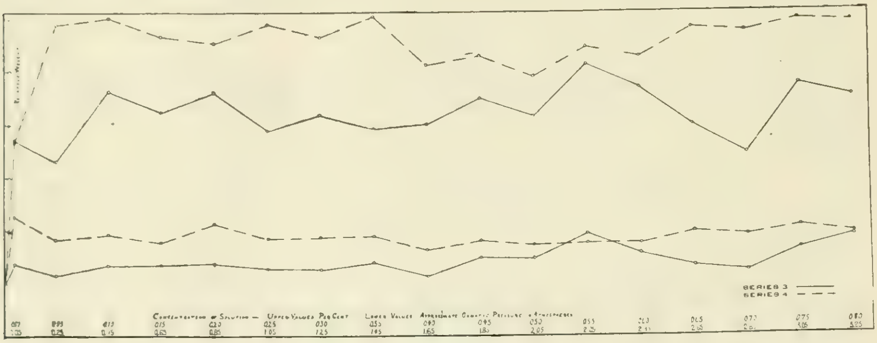

Figure 3. Graphs of dry weights of wheat plants, series 3 and 4. Upper graphs, tops; lower graphs, roots.

The relative dry weights given in table XV are shown as graphs in figure 3, where abscissae and ordinates are similar to those of figure 2. These graphs show that the trends of growth in the two series agreed well, but that this trend was somewhat more uniform in series 4 than in series 3. They also show that the medium containing di-potassium phosphate (series 3) produced equal growth of tops in solutions with from 0.45 to 3.25 atmospheres approximate osmotic pressure (0.1 to 0.8 per cent. concentration), while with mono-potassium phosphate as the source of phosphorus (series 4), equal growth occurred over a much wider range, *i.e.*, from a pressure of 0.25 atmospheres (0.05 per cent. concentration) to the upper concentration limit of the series (3.30 atmospheres or 0.8 per cent.). That the range of good growth extends much beyond the greatest concentration in these series seems unlikely, however, in view of the results of series 1 and 2, already given. The maximum relative yield of tops (5.11) in series

3, which was obtained with an osmotic pressure of 2.25 atmospheres (0.55 per cent. concentration), is 94.11 per cent. of the relative yield (5.43) obtained in series 4 with practically the same concentration, but is only 84.88 per cent. of the maximum yield (6.02) from series 4, which was obtained from the solution with osmotic pressure of 1.50 atmospheres (0.35 per cent. concentration). In the case of roots the maximum yield (1.94) of series 3, which was obtained with an osmotic pressure of 2.25 atmospheres (0.55 per cent. concentration), is 110.9 per cent. of the yield from series 4 with the same concentration of the medium, but is only 85.2 per cent. of the maximum yield of series 4 (2.28), the latter obtained with a pressure of 0.05 atmospheres (0.01 per cent. concentration).

Comparing the relative efficiencies of the two phosphates used in these two series of cultures, as seems justifiable from the similarity of all other factors influencing the growth of the plants, mono-potassium phosphate appears to be distinctly superior to the di-potassium salt. The former is, therefore, to be recommended for use with Knop's [65] formula. While no definite optimum point stands out on the graphs of series 4 (with mono-potassium phosphate) the uniformity of the ordinates of this graph between osmotic pressures of 0.25 and 1.50 atmospheres (0.05 and 0.35 per cent. concentration) seems to favor the choice of some concentration in that range as the probable general optimum for growth. Such a choice agrees well with the recommendations of Sachs [60], Nobbe and Siegert [64, *1*], Birner and Lucanus [66] and Nobbe [66] cited in Part I (pages 140, 142, 146 and 148). On the basis of the complete ranges of concentrations giving good growth in the several series, however, the solution having 2.50 atmospheres osmotic pressure (0.6 per cent. concentration) was chosen as the optimum concentration of nutrient medium for the succeeding and most important portion of the present culture work.

CULTURES IN NUTRIENT SOLUTIONS WITH VARIOUS PROPORTIONS OF THE FOUR SALTS, BUT WITH APPROXIMATELY CONTROLLED OSMOTIC PRESSURES.

General treatment.

It is clear from the foregoing sections, as well as from earlier work, that a complex salt solution prepared according to the formula of Knop [65] is eminently suited to the growth of plants in water cultures. It will be remembered that such a solution always contains the four main component salts in the proportions by weight, four parts of $Ca(NO_3)_2$ to one part of each of the other three salts. While the proportions of the constituents remain the same, the total salt content of the solution, *i.e.*, its total concentration, may of course be varied at will through alteration in the amount of water employed, in relation to the total weight of the seven parts of salts called for by the formula, and it is with such differences in percentage of total salt content,—as well as with the relative efficiencies for producing plant growth,—of the two potassium phosphates, that the present part has thus far had to do. It has been shown that the wheat plants of this study, under the conditions here employed, grow equally well in a series of Knop's solutions having a relatively broad range of total salt content, but the possible effect upon the plants of alterations in the relative proportions of the four component salts in the medium has not yet received attention in this paper. The present section deals with the problem thus suggested.

From the general survey of the earlier work on the growth of plants in nutrient solutions, given in Part I, it is obvious that some different proportions of the same salts, as well as several different salts, have frequently been tested in water cultures, but there appears never yet to have been carried out any experimental investigation that even approaches logical completeness, bearing upon the best proportions of nutrient salts for plant growth in liquid media. As a perusal of the literature brings out, there is no good evidence leading to the supposition that Knop's solution, for example, may not be markedly improved for plant growth by more or less pronounced alterations in the proportions of the four salts therein employed. The problem here brought up is very complex. As far as the nutrient solution is concerned, there are obviously three variable conditions that must be considered in this connection, (1) the total concentration (total salt content), (2) the chemical nature of the component salts and (3) the relative proportions (partial concentrations in the complex solution) of the different constituents. To study the influence upon plant growth, of even a small number of the infinite series of possible combinations of these three variable conditions, would clearly be a very difficult and time-consuming work and one for which the present status of the general problem of plant nutrition is hardly yet prepared. But a certain restricted portion of this problem may profitably be attacked. As has been indicated, the second of the variables just mentioned (the kinds of salt used) has received no attention in the present study, beyond the already reported experimentation on the relative efficiencies of the two potassium phosphates. The experiments have all dealt with Knop's solution, and the present section will concern itself only with that form of this solution which contains the mono-potassium phosphate as source of phosphorus. Thus, only four salts enter into consideration.

The relative proportions of these four salts, however, may of course be varied so as to give a very large series of solutions all of the same total salt content, and the total concentration of the solution characterized by any given set of proportions of its salts may likewise be varied to give another series of solutions, the latter differing neither in kinds of salts nor in their relative proportions, but merely in total concentration. For the following series of cultures it was decided to employ only three different values of the variable of total concentration, while a much larger number of values of the other variable were employed. Three series of cultures, designated as series 5, 6 and 7, were carried out with nutrient media having concentrations of 0.60, 0.01 and 2.00 per cent., respectively. These three percentage concentrations correspond approximately to osmotic pressures of 2.50, 0.05 and 8.15 atmospheres. The first value (0.60 per cent. or 2.50 atmospheres pressure) was selected arbitrarily from the results of the preliminary culture series and was taken to lie well within the range of concentrations giving optimum growth.[80] The concentration of series 6 was selected as having been shown in the preliminary cultures to lie considerably below the optimum; and also, because it may be considered to approximate the concentration of many natural soil solutions. Finally, the concentration of series 7 was chosen as having been found in the preliminary cultures to be well above the limit for the optimum growth of young wheat plants; and also because it presents conditions of concentration similar to those to which plants may often be subjected in alkali soils.

To obtain evidence bearing upon the influence upon the growth of the plants, of the relative partial concentrations of the four salts in the medium, at any single total salt concentration, it is obviously necessary to maintain as constant as possible all other influential factors. Each series of cultures was conducted simultaneously under the greenhouse conditions already described, so that the different cultures of each single series should be strictly comparable if their total salt concentrations were the same throughout the series. It was practically impossible to conduct the three series in question simultaneously, but it seems hardly possible that the differences between these series (as will appear later) may be at all due to conditions other than that of total concentration. The best growth relative to the control culture in each of these series has been compared with the growth in the corresponding concentration of Knop's solution relative to its control culture of series 2 or 4. It is evident, of course, that this comparison may not give the exact relative efficiency of the two nutrient solutions, since they were not employed at the same time.

Following the idea of Livingston [00], and of Breazeale [05], it is here supposed that the total concentration of the medium is effective to influence the growth of plants therein, through its power to develop osmotic pressure when in contact with the membranes of the plant. If the osmotic pressure of the solution is the total concentration variable mentioned above, it follows that the differently proportioned solutions of each of the three series about to be reported must have the same total concentration in the osmotic sense, *i. e.*, they must be so prepared that all may be of the same osmotic pressure.[81]

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[80] As already suggested, a somewhat lower concentration, e.g., 0.2 per cent., would agree better with the conclusions of earlier workers and might be more truly optimum. A series at this concentration should be included in future work.

The control of the osmotic pressure of such varied solutions has been dealt with already by Livingston [00] in his study of polymorphism in Stigeoclonium. Employing Knop's solution at concentrations where electrolytic dissociation of all the salts was assumed to be complete, the relative and absolute amounts of the salts were varied in a manner to avoid changing the osmotic pressure of the solution as a whole, by balancing a decrease of each salt to one-tenth of its normal amount with a sufficient increase in each of the other three salts to keep the osmotic pressure constant.

The procedure followed in the present work is an amplification of Livingston's method. Using as a basis the osmotic pressure of Knop's solution for each of the three concentrations above defined, the total osmotic pressure was divided into ten parts and distributed among the four salts in all the various proportions. Thus, each salt produced, in the different solutions of the same series, from one-tenth to seven-tenths of the total osmotic pressure of the solution. With reference to potassium nitrate, for example, this salt produced seven-tenths of the total pressure in one solution, six-tenths in three solutions, five-tenths in six solutions, four-tenths in ten solutions, three-tenths in fifteen solutions, two-tenths in twenty-one solutions and one-tenth in twenty-eight solutions. The manner in which the osmotic pressure is reproportioned on the basis of Knop's solution is shown by the data given in table XVI, which apply to series 5, about to be described.

These variations in the proportions of the salts, as just outlined, produced a total of eighty-four solution cultures, to which was added, in each series, a control culture in distilled water. In dealing with so large a number of cultures the employment of some diagrammatic arrangement is manifestly desirable, to express the relative composition of the various solutions concretely and to facilitate the designation of the individual cultures and the comparison of the yields obtained. For this purpose the following scheme, which is an amplification of the plan adopted by Schreiner and Skinner [10, *1* and *2*], has been used. The successive increments of any one of the salts may be represented on separate planes, each forming a triangular diagram, one of these being, however, only a single point. In this manner, six equilateral triangular diagrams and one point have been taken to represent the seven different proportions of mono-potassium phosphate, while the control culture of the series (in distilled water) is represented by a

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[81] Regarding the use of the term *osmotic pressure* in this sense, A. Findlay (Osmotic Pressure. London. 1913) well remarks (p. 3, footnote) "that the expression is, strictly speaking, incorrect. A solution does not, in itself, have any osmotic pressure; and the term is used, in a somewhat loose manner certainly, to denote the hydrostatic or mechanical pressure *which would be produced* if the solution were separated from the pure solvent by a membrane or septum which is permeable only to the solvent."

TABLE XVI

Partial and total concentrations of 0.6 per cent. Knop's solution and of the reproportioned solution having approximately the same osmotic pressure as the 0.6 per cent. Knop's solution (about 2.50 atmospheres*), 7/10 of this pressure produced by KH_2PO_4 and 1/10 by each of the remaining three salts (series 5).

			KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3	Total
Knop's solution having 0.6 per cent. concentration.....	Percentage concentration.		.0857	.0857	.3428	.0857	.6000
	Volume-molecular concentration......	Decimal fraction..	.0063	.0071	.0209	.0085	....
		Common fraction..	$\frac{m}{158.7}$	$\frac{m}{140.6}$	$\frac{m}{47.8}$	$\frac{m}{117.6}$	....
	Osmotic factor**.......		1.8	1.6	2.6	1.9	....
	Osmotic concentration, M***...............		.0113	.0114	.0543	.0161	.0931
Reproportioned solution having approximately the same osmotic pressure as 0.6 per cent. Knop's solution............	Tenths of total osmotic pressure produced by each salt............		7	1	1	1	10
	Osmotic concentration, M***...............		.0652	.0093	.0093	.0093	.0931
	Osmotic factor**.......		1.8	1.6	2.6	1.9	....
	Volume-molecular concentration......	Decimal fraction..	.0362	.0058	.0036	.0049	....
		Common fraction..	$\frac{m}{27.6}$	$\frac{m}{172.3}$	$\frac{m}{277.7}$	$\frac{m}{204.1}$	....
	Cubic centimeters of volume-molecular solution (m)**** of each salt, required for 1 liter of reproportioned nutrient solution..............		36.20	5.80	3.60	4.88	....

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*Calculated by interpolation, see data of series 3 and 4, page 187.

**The osmotic factor (see page 178) represents the number of particles per molecule resulting from dissociation. While this factor must alter to some extent for each salt as its proportion in the nutrient solution changes, this effect seems not to introduce a serious error; the average extreme variation of this kind for the four salts in series 5 is less than 8 per cent. of the factor here given, which is the one actually used.

***The osmotic concentration (see page 178) represents approximately the molecular concentration of a cane sugar solution having the same osmotic pressure as the nutrient solution in question. It is employed as multiplier in the upper part of the table and as divisor in the lower part.

****The stock solutions actually used in series 5 were molecular.

second point, the latter, of course, not really belonging to the series of triangular diagrams. The positions representing the different cultures on the diagrams are in accordance with the number of tenths of the total osmotic pressure produced by each of the three remaining salts. The diagrams of figure 4 bring out the details of this arrangement. On the largest triangle (T1) are represented all of the twenty-eight cultures having one-tenth of their osmotic pressure due to KH_2PO_4. The cultures represented on the lower row (R1) of this triangle all have one-tenth of their osmotic pressure derived from KNO_3, those on the second row (R2) have two-tenths due to KNO_3, and so on, till the single culture of the seventh row (R7) has seven-tenths of its total pressure due to this salt. The first position at the left of each row represents a culture (C1) having one-tenth of its pressure due to $Ca(NO_3)_2$, the second position (C2) represents a culture having two-tenths of its pressure derived from this salt, and so on to the opposite side of the triangle, the seven cultures of which (C1 to C7) have, respectively, from one-tenth to seven-tenths of their osmotic pressure due to the calcium salt. These seven cultures on the right side of the triangle are also characterized, in common, by having a single tenth of their pressure due to $MgSO_4$. Proceeding from right to left the osmotic proportion of $MgSO_4$ in the solutions increases in a manner quite similar to that shown for the proportion of $Ca(NO_3)_2$, proceeding in the opposite direction; thus the seven cultures (R1C1 to R7C1), all having one-tenth of their pressure produced by $Ca(NO_3)_2$, have from seven-tenths to one-tenth produced by $MgSO_4$. The various cultures of the remaining triangles of the series are plotted in a manner in all respects similar to that just outlined.

For ease in reference these triangular diagrams will be referred to by the abbreviations T1 to T7, corresponding to the tenths of the total osmotic pressure due to mono-potassium phosphate. The rows of cultures will be designated as R1 to R7, beginning at the potassium nitrate side of the diagram and proceeding toward the opposite apex, and the individual cultures will be designated as C1 to C7, from left to right. The control in distilled water will be referred to as DW. It will be readily seen that these triangles might be arranged in the form of a tetrahedron with T1 at the base and T7 at the apex. It seems, however, unnecessary to deal with the series by such spatial arrangement in the present discussion.

To determine the osmotic proportions of the salts in any given culture solution, it is only necessary to observe the numbers of its triangle, row and culture. Thus, culture T1R3C3, for example, being located in triangle 1, has one part of its pressure due to the phosphate, 3 parts to potassium nitrate (since it is in row 3) and 3 parts each to magnesium sulphate and calcium nitrate (since its position is equally distant from the left and right sides of the triangle, respectively). For the sake of additional clearness,

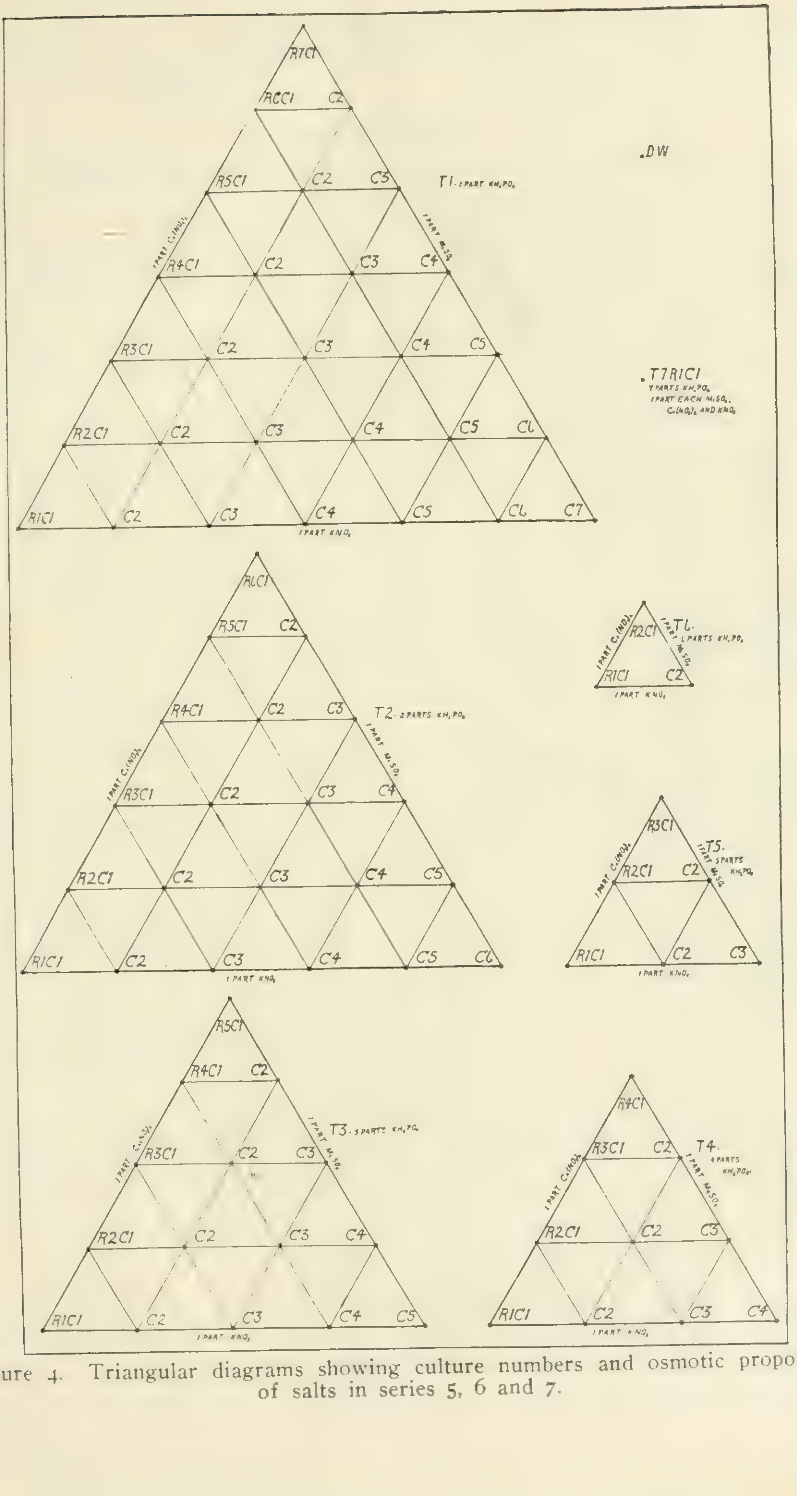

Figure 4. Triangular diagrams showing culture numbers and osmotic proportions of salts in series 5, 6 and 7.

since this method of dealing with such series of cultures is new, the osmotic proportions of the component salts for all of the solutions are given in table XVII.

TABLE XVII

Osmotic proportions of the four component salts in each of the 84 different culture solutions employed in series 5, 6 and 7 (see figure 4).

Culture No.	Number of tenths of total osmotic pressure derived from			
	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3
T1R1C1.	1	7	1	1
C2.	1	6	2	1
C3.	1	5	3	1
C4.	1	4	4	1
C5.	1	3	5	1
C6.	1	2	6	1
C7.	1	1	7	1
R2C1.	1	6	1	2
C2.	1	5	2	2
C3.	1	4	3	2
C4.	1	3	4	2
C5.	1	2	5	2
C6.	1	1	6	2
R3C1.	1	5	1	3
C2.	1	4	2	3
C3.	1	3	3	3
C4.	1	2	4	3
C5.	1	1	5	3
R4C1.	1	4	1	4
C2.	1	3	2	4
C3.	1	2	3	4
C4.	1	1	4	4
R5C1.	1	3	1	5
C2.	1	2	2	5
C3.	1	1	3	5
R6C1.	1	2	1	6
C2.	1	1	2	6
R7C1.	1	1	1	7
T2R1C1.	2	6	1	1
C2.	2	5	2	1
C3.	2	4	3	1
C4.	2	3	4	1
C5.	2	2	5	1
C6.	2	1	6	1
T2R2C1.	2	5	1	2
C2.	2	4	2	2
C3.	2	3	3	2
C4.	2	2	4	2
C5.	2	1	5	2
R3C1.	2	4	1	3
C2.	2	3	2	3
C3.	2	2	3	3
C4.	2	1	4	3
R4C1.	2	3	1	4
C2.	2	2	2	4
C3.	2	1	3	4
R5C1.	2	2	1	5
C2.	2	1	2	5
R6C1.	2	1	1	6
T3R1C1.	3	5	1	1
C2.	3	4	2	1
C3.	3	3	3	1
C4.	3	2	4	1
C5.	3	1	5	1
R2C1.	3	4	1	2
C2.	3	3	2	2
C3.	3	2	3	2
C4.	3	1	4	2
R3C1.	3	3	1	3
C2.	3	2	2	3
C3.	3	1	3	3
R4C1.	3	2	1	4
C2.	3	1	2	4
R5C1.	3	1	1	5
T4R1C1.	4	4	1	1
C2.	4	3	2	1

TABLE XVII—Continued

Culture No.	Number of tenths of total osmotic pressure derived from			
	KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3
T4R1C3.	4	2	3	1
C4.	4	1	4	1
R2C1.	4	3	1	2
C2.	4	2	2	2
C3.	4	1	3	2
R3C1.	4	2	1	3
C2.	4	1	2	3
R4C1.	4	1	1	4
T5R1C1.	5	3	1	1
C2.	5	2	2	1
C3.	5	1	3	1
T5R2C1.	5	2	1	2
C2.	5	1	2	2
R3C1.	5	1	1	3
T6R1C1.	6	2	1	1
C2.	6	1	2	1
R2C1.	6	1	1	2
T7R1C1.	7	1	1	1

As has been stated, the unit by which the four salts are apportioned to each solution is an osmotic one, being that amount of the salt in question which allows it to produce one-tenth of the total osmotic pressure of the complete solution. For example, the particular solution chosen for illustration in table XVI possesses seven of these units of KH_2PO_4 and one unit of each of the other three salts. Table XVI shows directly the volume-molecular values of the unit in question for the last three salts and the unit for KH_2PO_4 in this series is found by dividing the volume-molecular concentration there given (.0362) by 7. This gives 0.00517 as the volume-molecular concentration equivalent to one osmotic unit of this salt in this series. Table XVIII gives the values, in terms of volume-molecular concentration, of these osmotic units (osmotic tenths of the total salt content) for all four salts and for all three of the isosmotic series with which we are now dealing.

TABLE XVIII

Volume-molecular concentrations of osmotic units by which the four salts are apportioned in the different solutions of series 5, 6 and 7, the number of these units of each salt employed in each solution being shown in Table XVII.

Salt	Series 5	Series 6	Series 7
	m	*m*	*m*
KH_2PO_4	.00517	.0000643	.0169
$MgSO_4$	.00580	.0000940	.0199
$Ca(NO_3)_2$	.00360	.0000600	.0120
KNO_3	.00488	.0000900	.0163

To determine the volume-molecular partial concentration of any salt in any solution it is necessary merely to multiply the value given for its osmotic unit in table XVIII, by the number of units employed as shown in table XVII. The concentration of the stock solution by means of which the salts are actually measured out in preparing these culture media must of course be determined by convenience in measuring and by the total concentration of the complete solution required. In the present work, these stock solutions were volume-molecular (m) in the case of series 5, one-fiftieth volume-molecular $\left(\frac{m}{50}\right)$ in that of series 6, and one and one-half volume-molecular $\left(\frac{3m}{2}\right)$ in the case of series 7.

In preparing the four separate stock solutions for the present phase of the culture work, it seemed desirable that they be equal in molecular concentration. Their concentration was limited, however, by the solubility of mono-potassium phosphate. The latter salt forms a saturated solution slightly below a 1.75 volume-molecular concentration, for temperatures between 20° and 23° C., and a 1.5 volume-molecular solution develops a slight sediment after a few days at room temperatures, possibly as a result of action upon the glass of the container. It was therefore decided that a molecular solution of each salt be employed; this formed no precipitate after long standing, in any case.

Facility in handling these solutions was enhanced by connecting each stock-solution bottle, by means of a siphon, with the side arm of a 25 cc. burette provided with a two-way stop-cock, so that the burette could be refilled quickly after each measurement. The rubber tubing employed in these connections had been previously thoroughly soaked in the particular solution to be used and subsequently washed. A carboy of distilled water, also with siphon connection, located conveniently near the stock solutions served to expedite the filling of the 250 cc. standard flask employed in making dilutions and further to decrease the time consumed in preparing the nutrient solutions. The remaining details in the conduct of series 5, 6 and 7 were dealt with in the same manner as in the preliminary series, excepting that, in the present instances, the aerial conditions affecting transpiration were measured and recorded by means of the standard porous cup atmometer as already mentioned. The instrument was placed alongside the group of culture bottles in the experiment house.

Series 5 (Isosmotic solutions having approximately 2.50 atmospheres of osmotic pressure).

This series was grown in solutions of 0.6 per cent. concentration (approximately 2.50 atmospheres of osmotic pressure) during the 24 days from March 20 to April 13, 1913. During the first 14 days the cultures were arranged in seven rows of twelve jars each, the rows having an east-west direction, with the control at one side. Each morning the most southerly row was changed to the opposite side of the block of cultures, the entire block being moved correspondingly toward the south. On the fifteenth day this arrangement was replaced permanently by the triangular arrangement already described. On the sixteenth day of this series the strength of light was modified by application of white lead to the glass of the roof, thus rendering the use of cheesecloth shade unnecessary throughout the remaining culture work.

During the course of series 5 a small amount of flocculent, white or reddish-brown sediment appeared in all the nutrient solutions between changes. The amount of this was insufficient, however, to indicate serious disturbances of salt balance; moreover, such disturbance would be likely to produce crystalline rather than flocculent precipitate. Tests of most of the residual nutrient solutions at the last renewal showed acidity to litmus paper. The evaporation from a Livingston standard atmometer, showing the evaporating power of the air during the growth of series 5, gave a daily mean of 21.64 cc., a maximum daily rate of 26.40 cc. (on the thirteenth day of the period), a minimum daily rate of 7.58 cc. (on the twenty-second day), and a total loss from the instrument for the entire period (of twenty-four days) of 519.30 cc. Fumigation by tobacco was necessary on the eighteenth day in order to suppress an attack of black aphis. After 17 days of growth, dying of the basal leaves became noticeable; but this phenomenon did not greatly increase up to the time of harvesting. The tops of these plants were quite uniform in size after a period of 12 days. The roots, however, showed considerable differences, those of cultures T1R1C3, T1R3C3, T1R3C5, T1R5C3, T1R6C1, T3R1C2 and T3R2C1 being superior to the others, and those of cultures T1R1C1, T1R2C2, T1R2C3, T2R1C5, T2R3C3 and T4R1C3 being especially characterized by short secondary roots. After 19 days of growth the following cultures possessed especially long root systems: T1R6C1, T2R4C2, T2R4C3, T2R5C1, T3R2C1, T3R3C3, T4R1C1, T4R2C2, T5R1C1, T5R1C2, and T5R2C2.

After 12 days of growth a remarkable condition appeared in the tops of many of these cultures. In some of the cultures the tips of the leaves rolled backward laterally to form a stiff needle-like structure. This phenomenon was usually preceded by yellowing and contracting of the tissue at a point a few centimeters from the tip of the leaf, and the entire region above the contraction ultimately bleached to a greater or less extent. This form of injury gradually spread through the separate cultures and through a large part of the series. Finally, the young leaves just emerging from the sheath were affected in a somewhat different manner, these leaves rolling for most of their length, becoming limp and coiling spirally, either at the middle or toward the base, without notable discoloration. This condition frequently appeared in cultures where the older leaves were attacked but slightly or not at all and appeared to be a modification of the injury previously observed. Table XIX gives the results of surveys of this phenomenon at intervals of three or four days, the numbers showing how many leaves were affected in each culture at each survey. Severe and slight injury are separately represented. All of the cultures are given in the list, even though they may not have shown any injury.

TABLE XIX

Development of leaf injury in the plants of series 5

Number of days from beginning of culture..	12		16		19		23	
Culture number	Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T1R1C1............	3	2	6	1	14	1	15	1
C2............	..	..	3	..	3	3	10	1
C3............	..	..	..	..	..	..	2	1
C4............	..	..	..	..	..	..	..	2
C5............	..	..	..	..	..	..	..	1
C6............	..	..	..	..	..	..	..	..
C7............	..	..	..	..	..	..	..	..
T1R2C1............	3	1	4	1	13	..	15	1
C2............	..	..	1	..	1	4	3	6
C3............	..	..	..	..	..	1	1	3
C4............	..	..	..	..	..	..	..	3
C5............	..	..	..	..	..	..	..	..
C6............	..	..	..	..	..	..	..	..
T1R3C1............	5	..	5	..	10	..	14	..
C2............	..	..	1	..	1	2	2	6
C3............	..	..	..	..	..	..	2	2
C4............	..	..	..	..	..	..	..	1
C5............	..	..	..	..	..	..	..	..
T1R4C1............	3	3	5	1	9	3	10	6
C2............	..	..	..	..	..	3	1	7
C3............	..	..	..	..	..	..	1	3
C4............	..	..	..	..	..	..	1	..
T1R5C1............	2	1	3	..	8	..	8	..
C2............	..	..	..	..	..	1	4	..
C3............	..	..	..	..	..	..	..	1

TABLE XIX—Continued

Number of days from beginning of culture..	12		16		19		23	
Culture number	Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T1R6C1	1	..	1	..	5	1	11	..
C2	..	..	..	..	..	..	3	1
T1R7C1	..	..	..	..	1	2	6	..
T2R1C1	6	1	6	5	12	..	15	..
C2	..	1	..	3	..	3	4	6
C3	..	..	..	..	..	..	1	2
C4	..	..	..	..	..	..	1	..
C5	..	..	..	..	..	..	..	..
C6	..	..	..	..	..	..	..	..
T2R2C1	5	..	5	3	14	..	15	..
C2	..	..	..	..	1	2	3	3
C3	..	..	..	..	..	..	..	3
C4	..	..	..	..	..	..	..	..
C5	..	..	..	..	..	..	..	..
R3C1	6	..	6	5	11	..	17	..
C2	..	..	..	..	1	..	3	3
C3	..	..	..	..	..	..	..	2
C4	..	..	..	..	..	..	..	..
R4C1	1	2	2	1	6	3	10	4
C2	..	..	..	..	..	1	2	2
C3	..	..	..	..	..	..	..	..
R5C1	..	1	3	..	5	..	6	..
C2	..	..	..	..	..	2	1	1
R6C1	..	..	..	..	..	5	4	2
T3R1C1	3	1	3	5	12	..	13	1
C2	..	..	..	1	..	4	4	3
C3	..	..	..	..	..	..	1	2
C4	..	..	..	..	..	..	..	..
C5	..	..	..	..	..	..	..	..
R2C1	5	..	6	1	10	..	13	2
C2	..	..	..	..	..	..	..	3
C3	..	..	..	..	..	..	2	1
C4	..	..	..	..	..	..	..	..

TABLE XIX—Continued

Number of days from beginning of culture	12		16		19		23	
Culture number	Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury		Number of leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T3R3C1	3	2	4	1	11	..	8	7
C2	..	..	..	..	..	..	3	3
C3	..	..	..	..	..	..	..	.
R4C1	..	..	..	..	6	..	6	1
C2	..	..	..	..	..	..	..	2
R5C1	..	..	..	..	1	..	3	4
T4R1C1	2	1	3	8	3	8	4	9
C2	..	..	..	..	1	..	2	3
C3	..	..	..	..	..	..	1	2
C4	..	..	..	..	..	..	..	..
R2C1	..	1	1	6	4	7	9	..
C2	..	..	..	..	..	..	2	2
C3	..	..	..	..	..	..	..	..
R3C1	..	..	..	3	..	3	9	..
C2	..	..	..	..	..	..	1	1
R4C1	..	..	1	..	1	3	2	3
T5R1C1	..	2	1	7	5	3	12	1
C2	..	..	..	..	..	..	4	2
C3	..	..	..	..	..	..	..	..
R2C1	..	..	2	4	4	6	11	..
C2	..	..	..	..	..	..	..	4
R3C1	..	..	1	1	1	2	9	1
T6R1C1	..	..	..	4	1	3	7	1
C2	..	..	..	..	..	..	1	..
R2C1	..	..	..	..	..	..	6	..
T7R1C1	..	2	1	3	1	3	7	3

This injury appears to be related to the magnitude of the osmotic ratio of magnesium sulphate to calcium nitrate in the medium. In order most conveniently to appreciate this relation, reference may be had to table XX, which presents the cultures showing most severe injury, those having only one leaf injured, and those remaining uninjured, on the twenty-third day of the culture period, just preceding harvest, together with the corresponding values of the osmotic ratio. In this table the maximum ratio in each series is indicated by full face type and the minimum value by italics.

TABLE XX

Relation of leaf injury to the value of the osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$, in series 5.

Ten or more leaves severely injured		Only one leaf injured		No injury	
Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$	Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_3}$	Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$
T1R1C1	**7.0**	T1R1C5	0.60	T1R1C6	0.33
T1R1C2	6.0	T1R3C4	0.50	*T1R1C7*	*0.14*
T1R2C1	6.0	*T1R4C4*	*0.25*	T1R2C5	0.40
T1R3C1	5.0	T1R5C3	0.33	T1R2C6	0.17
T1R4C1	4.0	**T2R1C4**	**0.75**	T1R3C5	0.20
T1R6C1	*2.0*	T6R1C2	0.50	T2R1C5	0.40
T2R1C1	6.0			T2R1C6	0.17
T2R2C1	5.0			**T2R2C4**	**0.50**
T2R3C1	4.0			T2R2C5	0.20
T2R4C1	3.0			T2R3C4	0.25
T3R1C1	5.0			T2R4C3	0.33
T3R2C1	4.0			**T3R1C4**	**0.50**
T5R1C1	3.0			T3R1C5	0.20
T5R2C1	*2.0*			T3R2C4	0.25
				T3R3C3	0.33
				T4R1C4	0.25
				T4R2C3	0.33
				T5R1C3	0.33

It is clear from the data of table XX, as well as from a study of the relative positions of these cultures on the triangular diagrams (figure 4), that (1) the cultures characterized by the highest values of the osmotic ratio here considered were all severely injured, (2) that those with the lowest values (0.14 to 0.50) were free from injury, and (3) that a few with intermediate values (0.25 to 0.75) are indicated as showing some injury. Of course there is overlapping of the last two ratio ranges, but it is clear that no injury occurred with a value of this osmotic ratio lower than 0.25. It is therefore probably safe to conclude that this injury is not to be expected with osmotic ratios ($MgSO_4 : Ca(NO_3)_2$) below 0.25, and that osmotic ratios of 2.0 or above may be expected to produce very marked injury. The osmotic ratio of 0.25 is equivalent, in these solutions, to a molecular ratio of 0.40; that of 2.0 is equivalent to 3.23, molecularly.

It appears, furthermore, that the partial osmotic concentrations of the two remaining salts (KNO_3 and KH_2PO_4) in the medium determine the extent to which the intermediate values (0.25 to 0.75) of the magnesium-calcium ratio may produce the injury in question. Thus, the ratio value 0.25 produced injury in culture T1R4C4 and failed to do so in cultures T2R3C4, T3R2C4 and T4R1C4; the value 0.50 produced injury in cultures T1R3C4 and T6R1C2, but was not thus effective in cultures T2R2C4 and T3R1C4. The number of plants here employed was not large enough, however, to warrant more detailed discussion of the very complicated relations thus suggested. In connection with this matter of the influence of the proportions of the other salts on the optimum magnesium-calcium ratio, the sequel (data of series 6 and 7) will bring out the fact, not heretofore emphasized in the literature of this salt antagonism, that the value of this ratio limiting good growth alters with a change in the total concentration of the medium, probably even within the concentration range of from 0.05 to 0.8 per cent. (0.25 to 3.30 atmospheres) established as optimum by the preliminary cultures already discussed.

Undoubtedly the injury just considered is related to the effect of a physiological excess of magnesium over calcium to which Loew[82] and Loew and May[83] first directed serious attention. Loew[84] attributes the toxicity of magnesium to the displacement of calcium from protein compounds concerned with the synthesis of nuclei and chlorophyll bodies, while Hansteen[85] has found that calcium can antagonize an excess of magnesium only when supplied with the latter in the nutrient solution, its effect occurring at the absorbing surface of the root. Loew and his school, working chiefly with soils, have recommended the molecular ratio of magnesium to calcium of about 1 : 1 as best for the growth of cereals, with lower ratios for other plants. True and Bartlett [12] (page 154) have shown, however, that even in the case of peas the molecular ratio of 1 : 1 between magnesium and calcium determined maximum absorption by the plants when only the nitrates of these cations were employed in the nutrient solution. McCool,[86] who found calcium to be the most antitoxic cation of several employed by him in water cultures with peas, gives an extensive bibliography of investigations concerning the magnesium-calcium ratio. His own experiments showed that, in cultures composed of $\frac{n}{20}$ $CaCl_2$ with $\frac{n}{20}$ or any greater dilution of $MgCl_2$, the toxicity of magnesium was prevented by calcium. With $\frac{n}{200}$ $MgCl_2$, however, $CaCl_2$ at only $\frac{n}{1000}$ concentration was effective to prevent magnesium injury.

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[82] Loew, O., Die Bedeutung der Kalk- und Magnesiasalze in der Landwirtschaft. Landw. Versuchsst. **41:** 467-75. 1892. Page 474.

[83] Loew, O., and May, D.W., The relation of lime and magnesia to plant growth. U. S. Dept. Agric. Bur. Plant Ind. Bull. 1. 1901.

[84] Loew, O., The physiological role of mineral nutrients in plants. U. S. Dept. Agric. Bur. Plant Ind. Bull. 45. 1903. Page 49.

[85] Hansteen, B., Ueber das Verhalten der Kulturpflanzen zu den Bodensalzen. Jahrb. wiss. Bot. **47:** 289-376. 1910. Part II.

[86] McCool, M.H., The action of certain nutrient and non-nutrient bases on plant growth. Cornell Univ. Agric. Exp. Sta. Mem. **2:** 121-70. 1913.

The results brought out for series 5 of the present study show that molecular ratios of magnesium to calcium above 1.24 (osmotic ratio 0.5) are injurious to the variety of wheat here used during the first four weeks of its growth in solutions with a total concentration of 0.6 per cent. (2.50 atmospheres of osmotic pressure).

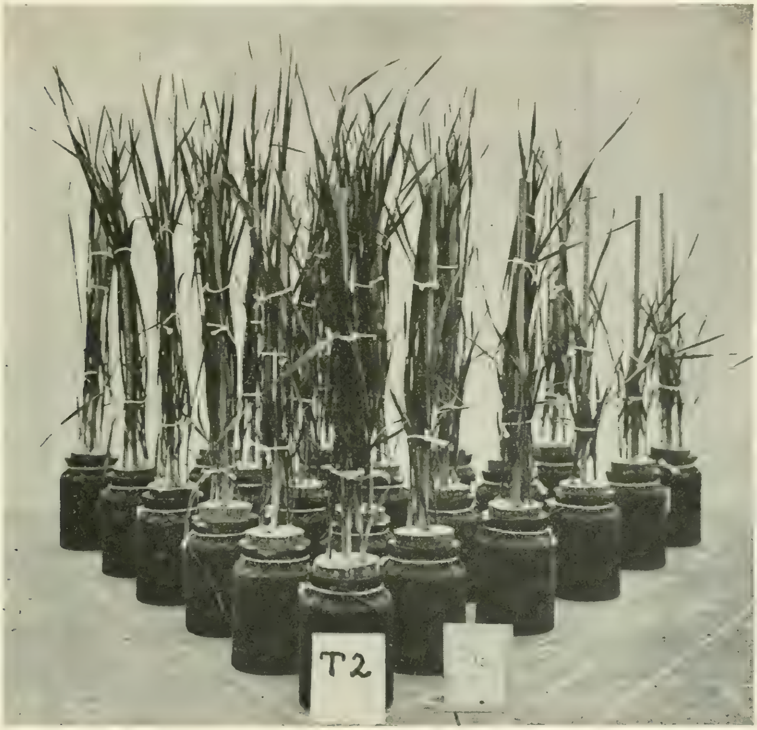

Figure 5. Triangle 2 of series 5, on twenty-third day. The culture nearest the observer has the highest content of KNO_3, that at the extreme right has the highest content of $MgSO_4$ (see also figure 4, for the arrangement).

On the twenty-third day of the period of these cultures photographs were taken of triangle 2 (figure 5) and of selected cultures from triangle 1 (figure 6), the former showing well the preponderance of magnesium injury about the apex of the triangle representing high partial osmotic concentration of $MgSO_4$, and the latter conveying some impression of the nature and general extent of the injury itself. In figure 5 the group of cultures is viewed from the apex with highest partial concentration of

KNO_3, the culture with highest concentration of $MgSO_4$ being at the extreme right.

Since the rate of water loss by transpiration, under any given complex of external conditions, is of considerable value as a measure of the extent of the leaf surface of the plants concerned, and since this measure has been extensively employed by other workers,[87] data of transpirational water loss were obtained for the last day of growth in this series, the cultures all being exposed to the same aerial conditions. Since the results obtained have their chief value when compared with corresponding data of the following series, they are here withheld and will be presented with the latter data after the discussion of the other series.

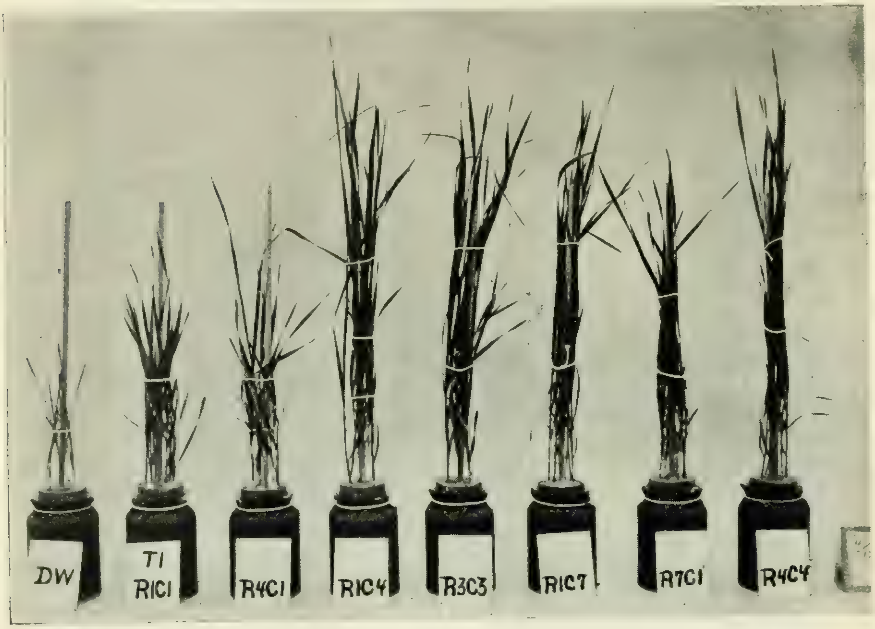

Figure 6. Selected cultures from triangle 1, series 5, on twenty-third day. The tips of the leaves in cultures R4C1 and R1C4 show characteristic magnesium injury.

Yields of Series 5. After 24 days of growth the cultures were harvested and the yields obtained in the usual manner. The six control plants had produced 0.1468 g. of oven-dried tops and 0.1132 g. of roots. To facilitate ready comparison of the yields from the various cultures their dry weights, relative to the control as unity, have been plotted on the triangular diagrams of figure 7, the upper number in the case of each culture position representing the weight of tops (relative to that of the control culture as unity) and the lower that of roots. The diagrams are similar in their

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[87] Livingston, [05], [07], and other later papers from the U. S. Bureau of Soils, etc.

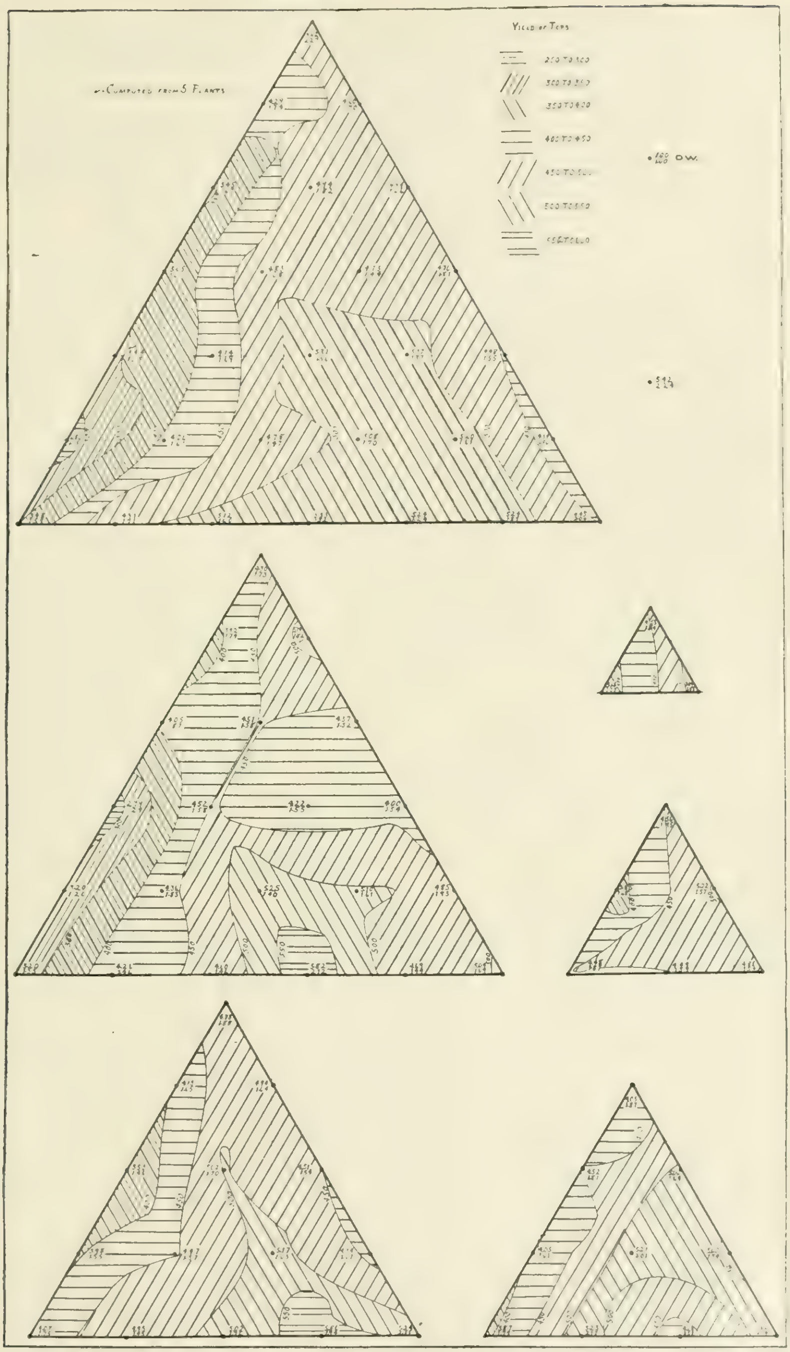

Figure 7. Charts of dry weights of tops and roots of cultures of wheat from series 5, six plants per culture, grown twenty-four days in nutrient solutions containing the salts of Knop's formula in varying proportions but equal in osmotic pressure. Total concentration, 2.46 atmospheres of osmotic pressure, or 0.6 per cent. The upper number in each position represents weight of tops, the lower that of roots. The different areas of the triangles, according to weight of tops, are shaded differently. (See also figure 4, for culture numbers and numbers of triangles.)

arrangement to those of figure 4. In order to differentiate the regions of the triangles with regard to yield of tops, each triangle has been divided into several partial areas by what may be called (in the absence of a better term) iso-yield lines. The areas thus formed have been differentiated by parallel-line shading on the graphs. This method has been followed also in the succeeding series, the values of the various shadings being given by legends on the corresponding figures.

Considered as a whole, the yields of series 5 show that the plants possessed considerable power of adaptation to wide variations in the proportions of nutrient salts. A comparison of the yields with the data of the survey of magnesium injury already given (table XX) shows that the lowest yields were associated with the same cultures as were characterized by the greatest injury.

The greatest dry weight of tops, 5.86 times that of the control in distilled water, occurred in culture T3R1C4, where the osmotic proportions of salts were as follows: $KH_2PO_4 : MgSO_4 : Ca(NO_3)_2 : KNO_3 = 3:2:4:1$. The lowest weight, 2.79 times that of the control, occurred in culture T2R3C1, where the osmotic proportions of salts, in the same order, were 0.7 : 1.3 : 0.3 : 1.0. The best growth of tops was, therefore, 2.10 times the poorest, according to this criterion of dry weight. In view of the fact that the yield of culture T2R1C4 was nearly equal to that from culture T3R1C4, being 5.82 times the control (with the proportions of the four salts, in the order just given, 2 : 3 : 4 : 1), and since these two best cultures occupy closely similar positions on adjacent triangles of the series, the average of their yields (5.84) relative to that of the control, seems to be justified as an optimum for this series. The average osmotic proportions of salts in the order already given is, 2.5 :2.5 :4.0 :1.0, for these two cultures; which corresponds to the molecular proportions, 2.66 :2.97 :2.95 :1.00. It is interesting to compare these data with the corresponding data of Knop's solution of 0.6 per cent. concentration (approximately 2.50 atmospheres of osmotic pressure) in series 4, which produced a yield relative to the control of 5.26, with the osmotic proportions of the salts, 0.76 :0.71 : 3.41 : 1.00; molecular proportions 0.76 : 0.85 : 2.53 : 1.00. This average of the two best yields may be considered as produced, therefore, by an interpolated optimum solution containing approximately only 0.7 as much $Ca(NO_3)_2$ and 0.6 as much KNO_3, but 2.1 times as much KH_2PO_4 and $MgSO_4$, on the molecular basis, as is contained in a Knop's solution of the same osmotic pressure.

At the particular total concentration of this series (0.6 per cent., 2.50 atmospheres osmotic pressure) the culture solution which produced the greatest dry weight of tops was, in this respect, somewhat superior to Knop's solution, having produced 11.0 per cent. greater yield relative to its control as unity. This superior yield corresponds to a derived optimum solution containing approximately only 0.7 as much $Ca(NO_3)_2$ and 0.6 as much KNO_3, but twice as much each of KH_2PO_4 and $MgSO_4$, on the molecular basis, as the Knop's solution of the same total concentration. While a high proportion of nitrate is generally recognized as promoting growth, it will be noted that nitrate was not the controlling factor in this case, for this ion is present in Knop's solution in much higher proportion than in the optimum solution derived from series 5. Considering the depression of yield in cultures with a high proportion of magnesium, one would hardly be led to infer that the superiority of the optimum solution in series 5 was due to its higher ratio of magnesium to calcium as compared with Knop's solution. The results of series 7, to be presented subsequently, show, however, that calcium may, under certain conditions, be even more injurious than magnesium. Moreover, further culture work has indicated that whether or not injury occurs, and probably also whether or not a superior yield is obtained, is determined by the complex of inter-influences among all the ions in solution. This view is supported by the work of Hansteen [10], who (with wheat seedlings), observed antagonism between several pairs of cations; and of Miyake,[88] who, (with rice seedlings), observed antagonism not only between cations, but also to a smaller extent, between anions. It may be stated also that McCool [13] found mutual antagonism between several pairs of cations, some of which were nutrient and others non-nutrient.

It is merely to be observed, therefore, that the best proportion of salts in series 5, which gave a yield superior to that from Knop's solution, contained higher proportions of magnesium, potassium, phosphate and sulphate, but lower proportions of calcium and nitrate, relative to all the other ions present, than did the latter solution.

The yield of roots was greatest from culture T1R7C1, 2.27 times that from the control, and least from culture T1R2C1, where it was 1.15 times that from the control. The former yield is 1.98 times the latter, which shows a slightly narrower range of root weights than that shown in the case of the weight of tops. Variations in yields of roots do not, however, correspond with those of tops. Thus, for example, culture T1R1C5 gave a yield of roots 1.24 times the control and of tops 5.24 times the control, while culture T1R7C1 with a high yield of roots, 2.27 times the control, yielded tops only 4.62 times the control. Apparently, therefore, the development of the tops of wheat, in water cultures such as were here employed, is not correlated with and proportional to the development of roots in the manner which Livingston[89] found to obtain for this plant when grown in the same soil with different additions. This difference seems to be explainable by the supposition that, while in liquid media, the concentrations of the various molecules and ions are not greatly influenced by the metabolism of the plant, and considerable differences in root area may have slight influence upon the growth of tops, in soil cultures, on the other hand, the activities of the plant may (through excretion of carbon dioxide, etc.), alter the concentration of the soil solution in various ions, and the extent of the root system may then become a much more important factor in the growth of tops.

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[88] Miyake, K., The influence of salts common to alkali soils upon the growth of the rice plant. Jour. Biol. Chem. **16**: 235-63. 1913.

[89] Livingston, B.E., Note on the relation between the growth of roots and of tops in wheat. Bot. Gaz. **41**: 139-43. 1906.

For the optimum yield of roots the average of the yields from cultures T1R7C1 and T7R1C1 may be taken, which is 2.25, relative to the corresponding yield of the control, with the osmotic proportions of salts, 1.00: 0.25: 0.25: 1.00 (molecular proportions, 3.40: 0.68: 0.43: 1.00). This average is 29.1 per cent. greater than the yield from Knop's solution (1.75 relative to the control as unity) in series 4 at the same osmotic concentration, a superiority over the latter solution nearly three times as great as was brought out in the case of tops.

Series 6 (Isosmotic solutions having approximately 0.05 atmospheres of osmotic pressure).

This series of cultures grew during the 24-day period from April 4 to April 28, 1913, in solutions of 0.01 per cent. total salt concentration (approximately 0.05 atmospheres of osmotic pressure). The stock solutions employed in the preparation of the cultures had a concentration of *m*/50. The manner in which the osmotic pressure of 0.01 per cent. Knop's solutions was determined and redistributed is shown by the data of table XXI, in which the items correspond throughout with those already explained in connection with series 5 (table XVI).

In this series the seedlings employed were somewhat older than usual; they were six days old and were 6 cm. high when placed in the culture jars with distilled water, and they remained in the latter two days before being transferred to the nutrient solutions. The culture jars were arranged in triangles on the greenhouse table throughout the period of the culture. The evaporation from a Livingston standard atmometer, showing the evaporating power of the air during the period of the series, gave a daily mean of 19.61 cc., a maximum daily rate of 30.04 cc. (on the eighteenth day of the period), a minimum daily rate of 5.62 cc. (on the twenty-fourth day) and a total loss from the instrument for the entire period (24 days) of 470.60 cc.

Both tops and roots were elongated and slender in this series. More noticeable differences in this respect appeared among the roots than among the tops, the shorter roots predominating where the culture solutions were lowest in content of magnesium sulphate and where the maximum content of potassium nitrate was present. After 22 days of growth the following

TABLE XXI

Partial and total concentrations of 0.01 per cent. Knop's solution and of the reproportioned solution having approximately the same osmotic pressure as the 0.01 per cent. Knop's solution (about 0.05 atmospheres*), 7/10 of this pressure produced by KH_2PO_4 and 1/10 by each of the remaining salts (series 6).

			KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3	Total
Knop's solution having 0.01 per cent. concentration.....	Percentage concentration.		.00143	.00143	.00572	.00143	.01000
	Volume-molecular concentration......	Decimal fraction..	.00011	.00012	.00035	.00014	....
		Common fraction..	$\frac{m}{9524}$	$\frac{m}{8403}$	$\frac{m}{2865}$	$\frac{m}{7092}$	....
	Osmotic factor**.......		2.9	2.0	3.0	2.0	....
	Osmotic concentration, M***..............		.00030	.00024	.00105	.00028	.00187
Reproportioned solution having approximately the same osmotic pressure as 0.01 per cent. Knop's solution.............	Tenths of total osmotic pressure produced by each salt............		7	1	1	1	10
	Osmotic concentration, M***...............		.00130	.00019	.00019	.00019	.00187
	Osmotic factor**.......		2.9	2.0	3.0	2.0	....
	Volume-molecular concentration......	Decimal fraction..	.00045	.00009	.00006	.00009	....
		Common fraction..	$\frac{m}{2222}$	$\frac{m}{11111}$	$\frac{m}{16667}$	$\frac{m}{11111}$	....
	Cubic centimeters of volume-molecular solution (m)**** of each salt, required for one liter of reproportioned nutrient solution..............		0.451	0.094	0.062	0.094	....

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*Calculated by interpolation, see date of series 3 and 4, page 187.

**The osmotic factor (see page 178) represents the number of particles per molecule resulting from dissociation. While this factor must alter to some extent for each salt as its proportion in the nutrient solution changes, this effect seems not to introduce a serious error; the average extreme variation of this kind for the four salts in series 5 is less than 8 per cent. of the factor here given, which is the one actually used.

***The osmotic concentration (see page 178) represents approximately the molecular concentration of a cane sugar solution having the same osmotic pressure as the nutrient solution in question. It is employed as multiplier in the upper part of the table and as divisor in the lower part.

****The stock solutions actually used in series 6 were $\frac{m}{50}$.

cultures possessed especially long roots, those marked with an (e) being of extreme length: T1R1C1(e), T1R1C2(e), T1R1C6, T1R2C1(e), T1R6C2, T2R1C1, T2R1C2(e), T2R2C1(e), T2R3C2(e), T5R1C3(e), T6R2C1(e).

After 12 days of growth one young, third leaf of culture T1R4C1 exhibited an incipient stage of the characteristic magnesium injury observed in series 5. While this one case developed to a severe stage, no further trace of the injury became visible in this series, which suggests that this single instance of injury may have been due to a specific weakness or susceptibility of the particular leaf involved.

After 14 days of growth the plants appeared to be of paler color than those of previous series and showed a tendency to weakness of stem, general flaccidity and inequality of vigor. After 16 days, yellowing and subsequent dying of the basal leaves appeared in a few cultures irregularly distributed throughout the triangles. The latter phenomenon became more rapidly pronounced toward the close of the period of growth. After 22 days of growth the data given in table XXII, regarding this phenomenon of yellowing and dying of the leaves, were obtained, the cultures being here named in the decreasing order of the seriousness of the diseased condition, the very severe cases being marked with an (s).

TABLE XXII

Survey of yellowing and death phenomena in series 6

Severe injury	Slight injury	Severe injury	Slight injury	Severe injury	Slight injury	Severe injury	Slight injury
T1R1C1	T1R3C2	T2R1C1	T2R1C3	T3R1C1	T3R1C3	T5R1C1 (s)	T5R3C1
R1C2	R2C3	R1C2	R3C1	R2C1	R4C1	R1C2 (s)	R1C3
R2C1	R2C4	R2C1	R4C1	R1C2	R2C3	R2C1	
R3C1	R5C1	R2C2	R3C2	R3C1		R2C2	
R4C1	R6C1		R2C3	R2C2			
R1C3	R1C5		R1C4			T6R1C1 (s)	
R2C2			R1C5	T4R1C1	T4R1C3	R2C1 (s)	
			R2C4	R2C1	R4C1	R1C2 (s)	
			R1C6	R1C2	R1C4		
				R2C2		T7R1C1 (s)	
				R3C1			

From the data of table XXII it is apparent that the phenomenon in question was most pronounced in the smaller triangles and about those apices of the larger ones where the partial concentration of magnesium sulphate was highest. Since the culture solutions of these regions were comparatively low in partial concentration of the nitrate radicle, it appears highly probable that the yellowing and premature death here considered were due to lack of nitrate. It is well known that a lack of this radicle hastens maturity in plants while an excess prolongs the growth period.

Data of the transpiration of selected cultures from this series will be presented later, in connection with similar data from series 5 and 7.

On the twenty-third day of growth, photographs were taken of triangle 2 and of selected cultures from triangle 1, and these are reproduced as figures 8 and 9. In figure 8 the triangular group of cultures is viewed from the apex having the highest partial concentration of KNO_3, the culture with the highest partial concentration of $MgSO_4$ being at the extreme right. These figures show the general condition of the plants and, especially, the absence of the peculiar magnesium injury which was so prevalent in series 5.

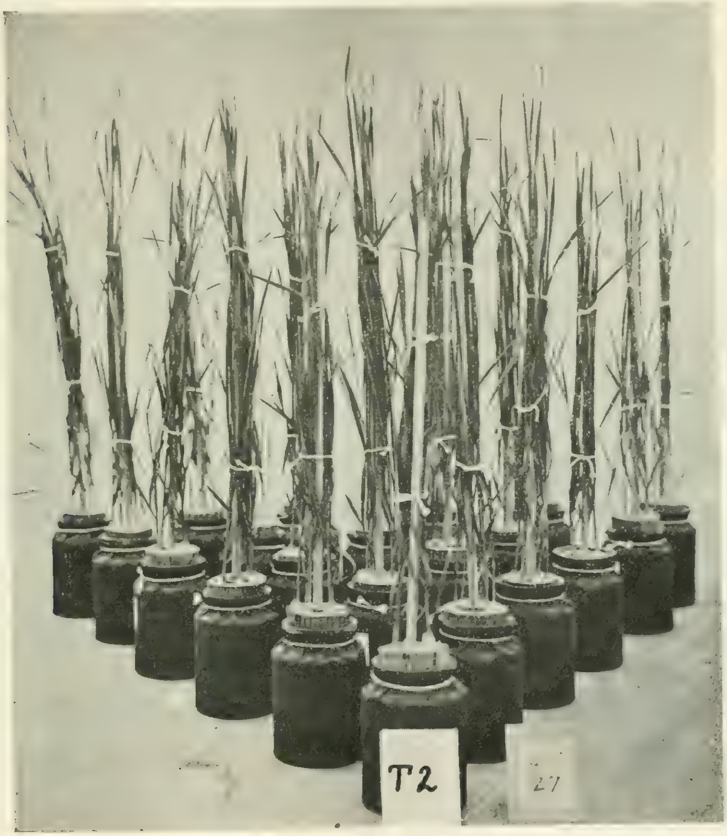

Figure 8. Triangle 2 of series 6, on twenty-third day. The culture nearest the observer has the highest content of KNO_3, that at the extreme right has the highest content of $MgSO_4$. (See also figure 4 for the arrangement.)

Yields of series 6. After 24 days of growth the cultures were harvested and treated in the usual manner. The six control plants produced a yield of 0.1412 g. of oven-dried tops and 0.0952 g. of roots. The yields, relative to the control as unity, have been plotted on the triangular diagrams of figure 10, where the upper values correspond to tops and the lower ones to roots. These diagrams are quite similar in form to those of figure 7 and are comparable thereto.

In the graphical arrangement of the yield of tops this series of cultures shows two prominent features: (1) the localization of the highest yields about the triangle apices at the right (regions having high content of calcium nitrate) and along the right sides of the triangles (characterized by low content of magnesium sulphate); (2) the restriction of lowest yields to the regions having high content of magnesium sulphate (left triangular apices) and along the left sides of the triangles, with low content of calcium nitrate. In these respects, this series resembles series 5, growth being accelerated by the lower ratios of magnesium to calcium in the medium and depressed by the higher ones. Furthermore, the yields of cultures similarly located in the several triangles of this series decrease markedly from the largest to the smallest triangle, that is, from the low to the high partial concentrations of mono-potassium phosphate. Apparently this depression of growth, which accompanied early maturity, as previously observed, was due, like the latter phenomenon, to low partial concentrations of nitrate.

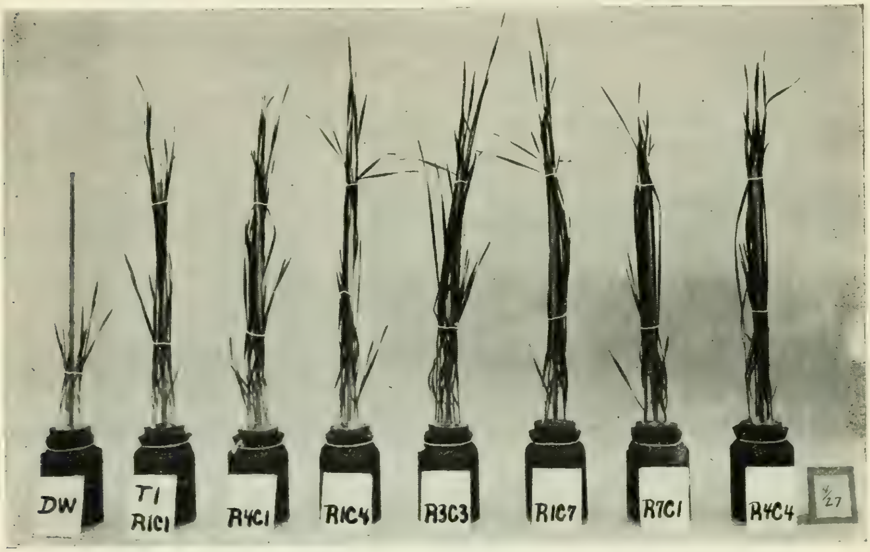

Figure 9. Selected cultures from triangle 1, series 6, on twenty-third day.

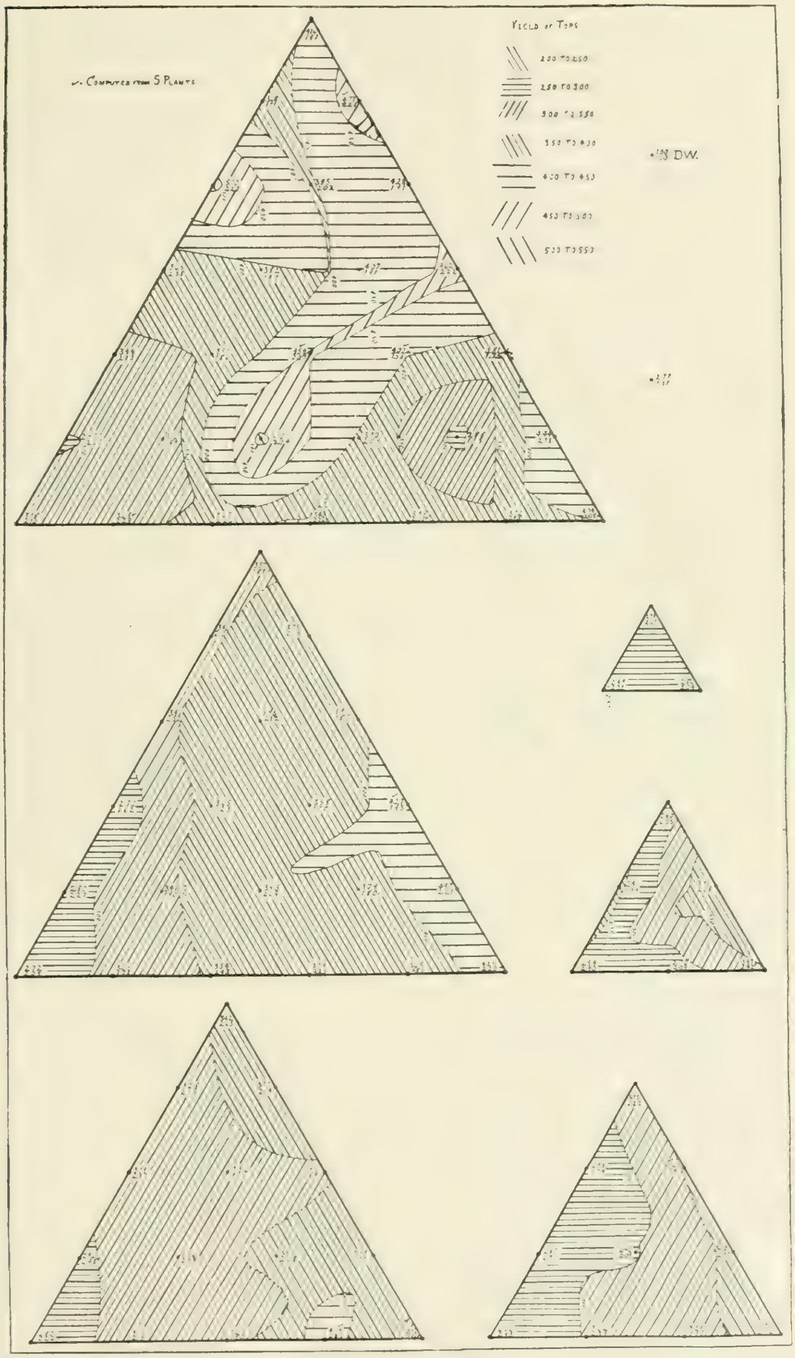

Figure 10. Charts of dry weights of tops and roots of cultures of wheat from series 6, six plants per culture, grown twenty-four days in nutrient solutions containing the salts of Knop's formula in varying proportions but equal in osmotic pressure. Total concentration, 0.05 atmospheres of osmotic pressure, or 0.01 per cent. The upper number in each position represents weight of tops, the lower that of roots. The different areas of the triangles, according to weight of tops, are shaded differently. (See also figure 4, for culture numbers and numbers of triangles.)

The greatest dry weights of tops in series 6 are from cultures T1R2C3 and T1R5C1, which produced yields relative to the control of 5.10 each, which is equivalent to 88.54 per cent. of the optimum, in this respect, for series 5. Since, however, the surrounding cultures do not harmonize with culture T1R5C1, the high yield of the latter seems to have been accidental, and the first culture named (T1R2C3) may be considered as the optimum culture solution of the series. The osmotic proportions of the nutrient salts in this culture solution are, $KH_2PO_4 : MgSO_4 : Ca(NO_3)_2 : KNO_3 = 0.5 : 2.0 : 1.5 : 1.0$ (molecular proportions, 0.34 : 2.00 : 1.00 : 1.00). The poorest relative yield (2.41) in this series was obtained from culture T5R2C1, with osmotic proportions of salts, in the preceding order, 2.5 : 1.0 : 0.5 : 1.0. Therefore, the best growth in the series may be considered as 2.12 times the poorest, a value practically identical with the corresponding one for series 5 (2.10, see page 208). Knop's solution with a concentration of 0.01 per cent. (0.05 atmospheres osmotic pressure) in series 4 produced a yield, relative to that of the control, of 3.67, with the osmotic proportions of salts, $KH_2PO_4 : MgSO_4 : Ca(NO_3)_2 : KNO_3 = 1.07 : 0.87 : 3.74 : 1.00$ (molecular proportions, 0.76 : 0.85 : 2.53 : 1.00). Therefore, the optimum culture of series 6 may be considered as producing 39.0 per cent. more growth of tops than did Knop's solution at the same concentration, a superiority of the new solution nearly four times as great as that (11.0 per cent., see page 208) obtained in the previous series, the latter with a much higher total concentration of the medium. This superior yield occurred with a culture solution containing approximately 0.6 as much KH_2PO_4, 3.1 times as much $MgSO_4$, 0.5 as much $Ca(NO_3)_2$ and 1.4 times as much KNO_3 as was contained in the Knop's solution of the same total concentration. The new solution thus contained considerably higher proportions of magnesium and sulphate but lower proportions of calcium and nitrate than occur in the corresponding Knop's solution.

Contrary to the indications of the general phenomena of growth in these cultures, the simple requirement of the plants for nitrate does not seem to have been the controlling factor in determining the optimum proportions of salts as shown by dry weight of tops; the injurious effect of a high partial concentration of calcium nitrate at this concentration seems to have thrown the optimum culture solution into a region considerably higher in magnesium sulphate and in potassium nitrate, but lower in potassium phosphate, than was the case in series 5.

In the yields of roots from this series there was much greater uniformity and a considerably higher average than in the case of series 5; in fact, the most striking characteristic of series 6 was the prevalent vigor and length of the roots. The highest relative dry weight of roots (2.63) was produced by culture T3R5C1, in which the proportions of salts, in the usual order, were, 0.6:0.2:0.2:1.0 (molecular proportions, 0.41:0.20:0.13:1.00). Knop's solution of the same total concentration produced (in series 4) a relative yield of roots of 2.28; the former yield may therefore be considered as 15.35 per cent. greater than the latter; but this superiority was only about 40 per cent. of that just brought out in the case of the dry weight of tops. Culture T1R2C3, which produced the greatest dry weight of tops, produced a relative yield of roots of 2.04, or only 90 per cent. of that from the corresponding Knop's solution (2.28, see page 187). The lowest dry weight of roots (1.15, relative to that from the control) occurred in culture T1R2C3. Thus the greatest weight of roots was 2.29 times the smallest, a ratio a little higher than that (2.12) deduced from the yields of tops. Although the optimum culture of this series, with reference to yield of roots, had a high proportion of potassium phosphate as compared with that in Knop's solution, the other cultures with high partial concentrations of this salt were not uniformly efficient in producing root growth. Neither were high yields of roots uniformly associated with high proportions of nitrate in the culture solutions.

As in the case of series 5, the dry weights of tops here bear no definite relation to those of roots. Thus, for example, culture T1R2C3, with a yield of tops of 5.10, produced a relative yield of roots of only 2.04, while culture T5R1C1, with a yield of tops of only 2.54, produced a relative yield of roots of 2.40. This condition seems rather surprising in view of the low total concentration of the nutrient solution of the present series, which might here tend to render the extent of the root surface a more important factor in the absorption of nutrient salts, and hence in the growth of tops, than is the case with more concentrated media.

Series 7 (Isosmotic solutions having approximately 8.15 atmospheres of osmotic pressure).

This series of cultures was carried out in solutions of 2.0 per cent. concentration (8.15 atmospheres of osmotic pressure) during the 24-day period from April 28 to May 22, 1913. The seedlings were six days old and about 7 cm. high, when transferred to the nutrient solutions.

The evaporation record from a standard atmometer during the growth of this series showed a daily mean of 16.49 cc., a maximum daily rate of 64.10 cc. (on the sixth day of the period), a minimum daily rate of 4.30 cc. (on the nineteenth day), with a total loss from the instrument for the entire period of 395.81 cc. It will be seen that this is the lowest mean evaporating power of the air to which any of these three series were subjected, series 5 having been exposed to the greatest. This period also shows the greatest fluctuation in this climatic factor, for the ratio of the daily maximum to the daily minimum was here 14.9, while the corresponding ratios were, for series 5 and 6, 3.5 and 5.3, respectively. The range of fluctuation in the conditions affecting transpiration was, therefore, much greater for these plants than for those of the two previous series.

Because of the high concentration of the solutions used in this series it was necessary to employ stock salt solutions at the highest possible concentration. The latter were prepared, therefore, with 1.5 molecular concentration. They were renewed after each two changes of the culture solutions, to avoid serious precipitation or other chemical readjustment. This made necessary four separate preparations of each of the four solutions. During the three or four days in which these stock solutions necessarily remained standing, between renewals of the culture solutions, sediments accumulated to a slight extent in the cases of magnesium sulphate and mono-potassium phosphate, more appreciably in the latter. These sediments were not disturbed, however, in removing the supernatant solutions; their amounts could not have sufficed to produce any considerable change in the concentration of the solutions. The method by which the redistribution of osmotic pressure was calculated for the several cultures of series 7 is given in table XXIII, in which the items correspond to those of tables XVI and XXI (pages 193 and 211) for series 5 and 6.

The following nutrient solutions of this series formed appreciable precipitates after the three-day period allowed between the usual changes, those marked with an (e) being excessive: T1R1C2, T1R1C3(e), T1R1C4(e), T1R1C5(e), T1R1C6(e), T1R2C2(e), T1R2C3(e), T1R2C4(e), T1R2C5, T1R3C3, T1R3C4, T2R1C2, T2R1C3, T2R1C4(e), T2R1C5, T2R2C3, T2R2C4, T3R1C3, and T3R1C4. These precipitates appeared to form independently of the growth of the plants, and hence these solutions are to be considered as unsuited for use in such culture work as this. On standing at about 21° C. without plants growing in them, solutions T1R1C4 and T1R1C5 precipitated first, the former after about 1 hour 22 minutes and the latter after about 1 hour 18 minutes. The amount of precipitate thus formed indicated that the former solution would limit (by its own instability and independently of the influence of the growing plants) the concentration at which this series could be employed without precipitation in the solutions. This solution was prepared, therefore, in concentrations ranging from 0.6 to 1.8 per cent. and allowed to stand at temperatures of from 19.0° to 22.5° C. A precipitate formed after two days in a solution of 1.2 per cent. concentration, but not after six days in one of 1.1 per cent. concentration. This shows that the limit of concentration to which the unplanted solutions of this series can be extended, at greenhouse temperatures, is about 1.0 per cent. In series 7, however, the full usual number of cultures was employed, at 2.0 per cent. concentration, in order to include in the results the effects upon the plants of the disturbance brought about by precipitation.

TABLE XXIII

Partial and total concentrations of 2.0 per cent. Knop's solution and of the reproportioned solution having approximately the same osmotic pressure as the 2.0 per cent. Knop's solution (about 8.15 atmospheres*), 7/10 of this pressure produced by KH_2PO_4 and 1/10 by each of the remaining salts (series 7).

			KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3	Total
Knop's solution having 2.0 per cent. concentration.....	Percentage concentration.		0.286	0.286	1.144	0.286	2.000
	Volume-molecular-concentration......	Decimal fraction..	.0210	.0237	.0697	.0283	....
		Common fraction..	$\frac{m}{47.62}$	$\frac{m}{42.19}$	$\frac{m}{14.35}$	$\frac{m}{35.35}$	....
	Osmotic factor**.......		1.81	1.53	2.55	1.87	....
	Osmotic concentration, M***...............		.0380	.0363	.1777	.0529	.3049
Reproportioned solution having approximately the same osmotic pressure as 2.0 per cent. Knop's solution............	Tenths of total osmotic pressure produced by each salt............		7	1	1	1	10
	Osmotic concentration, M***...............		.2135	.0305	.0305	.0305	.3049
	Osmotic factor**.......		1.81	1.53	2.55	1.87	....
	Volume-molecular concentration......	Decimal fraction..	.1180	.0199	.0120	.0163	....
		Common fraction..	$\frac{m}{8.47}$	$\frac{m}{50.25}$	$\frac{m}{88.33}$	$\frac{m}{61.35}$	....
	Cubic centimeters of volume-molecular solution (m)**** of each salt, required for one liter of reproportioned nutrient solution..............		118.0	19.9	12.0	16.3	....

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*Calculated by interpolation, see data of series 3 and 4, page 187.

**The osmotic factor (see page 178) represents the number of particles per molecule resulting from dissociation. While this factor must alter to some extent for each salt as its proportion in the nutrient solution changes, this effect seems not to introduce a serious error; the average extreme variation of this kind for the four salts in series 5 is less than 8 per cent. of the factor here given, which is the one actually used.

***The osmotic concentration (see page 178), represents approximately the molecular concentration of a cane sugar solution having the same osmotic pressure as the nutrient solution in question. It is employed as multiplier in the upper part of the table and as divisor in the lower part.

****The stock solutions actually used in series 7 were $\frac{3\,m}{2}$.

Pathological conditions appeared in these cultures at an early stage and developed in several forms during the period of the series. After only three days of growth, retardation of root development was decidedly apparent and the vigor of the tops in the individual cultures was not uniform, suggesting that development was controlled by specific physiological characters of the different individual plants. In view of this consideration, the replacing of weak and backward plants was abandoned when one plant had been replaced in each of cultures T1R1C3, T1R2C3, T1R4C1, T2R4C1 and T3R2C2, after three days of growth.

Further prominent evidences of disturbed growth in this series included: (a) wilting of the leaves, followed by death of the plants; (b) yellowing of the basal leaves, followed by death; (c) foliar striping, due to bleaching of the tissue between the leaf veins, and (d) magnesium injury. Moreover, in addition to the preceding phenomena, the salts crept by crystallization over the stoppers and up the stems of the plants in some cases, especially in cases where the highest proportions of potassium nitrate occurred. This phenomenon did not appear to interfere seriously with growth, however.

As was to be expected, root growth throughout this series was greatly depressed, undoubtedly as a result of the high total concentration of the solutions and its dependent osmotic effects. There was much variation in this condition, however. The poorer root systems were mere stubs of their primary axes. In some cases of conspicuously poor systems, the roots bent after entering the solution, in such manner that they formed little contact with the latter, this condition being especially noticeable in cultures T1R1C6 and T1R2C5. As growth progressed the better and poorer root systems became more sharply differentiated. It was noted that the root systems possessed few secondary roots and were dark colored in cultures T1R1C7, T1R2C6, T2R1C6 and T2R3C4, after eighteen days of growth.

In some of the cultures more or less of a compact columnar coating of white salt crystals, generally arranged perpendicularly to the axis, formed upon the roots. These crystals appeared under the microscope like the calcium sulphate crystals identified in the work of Part 2. After 18 days of growth the following cultures were characterized by exceptionally good root systems, those indicated by the letter (d) bearing the deposit just described: T1R1C1, T1R1C2(d), T1R2C2(d), T1R3C2, T1R4C2, T1R5C1, T1R5C2, T1R6C1, T1R6C2, T1R7C1, T2R1C1, T2R1C4, T2R1C5, T2R1C6, T2R2C1, T2R2C2, T2R2C3, T2R2C4, T2R3C1, T2R4C2, T2R5C1, T2R5C2, T3R1C1, T3R1C2, T3R2C1, T3R2C2, T3R3C2 and T3R4C1. At the same time the following cultures showed the poorest roots, the presence of crystals being again indicated as above: T1R1C3(d), T1R1C4(d), T1R1C5(d), T1R1C6(d), T1R2C3(d), T1R2C4(d), T1R2C5, T1R2C6, T1R3C4(d), T1R4C4, T2R1C6, T2R3C3, T2R3C4, T2R4C3, T2R6C1, T3R1C3(d), T3R1C4(d), T3R3C1, and the remaining cultures of the series from T3R5C1 to T7R1C1 inclusive, with the exception of T4R2C2, T5R1C3 and T5R2C2.

It is thus brought out that one-third of the cultures possessed comparatively good root systems. This was due to uniform development of the secondary roots rather than to general elongation at all comparable with that of the roots in series 5 and 6. On the other hand, nearly one-half of the cultures possessed poor root systems, as compared with the average root system of the present series. Most striking among the conditions apparently here related to root development are the proportions of calcium and of magnesium to the total salt content of the nutrient medium. In the cultures of triangles 4, 5 and 6, the characteristic poor root development cannot be due to high total concentration of the nutrient solution alone, for this concentration is the same throughout the entire series. It seems probable that this retardation of root growth was due partly to the strongly acid reaction produced by the high proportions of monopotassium phosphate in these cultures. In the cultures of triangle 1 the good root systems are confined to media in which one-tenth or two-tenths of the total osmotic pressure is due to calcium nitrate, and they are predominant in the vicinity of the triangle apex where the proportion of potassium nitrate is high. This appears to indicate the favorable influence of a low proportion of calcium and a high proportion of nitrate. In triangle 2 the good roots occur in no special region, while in triangle 3 their growth is again accelerated by a low partial concentration of calcium nitrate. It appears to be clear that in culture solutions of high total concentration, where the latter itself becomes a critical factor, the growth of roots, while undoubtedly favored by several different proportions of the nutrient salts, is most benefited by a low proportion of calcium nitrate. A large number of the poor root systems of triangle 1 were affected by the accumulation of a precipitate upon these organs. This condition must have affected root development directly, but it must also have been attended by disturbance of the proportions and of the actual amounts of salts in the nutrient solution, which might react unfavorably toward growth. In triangles 2 and 3 the poor root systems are located along the side where the proportion of magnesium sulphate is low, in triangle 4 only the central culture escaped depression of root development and in triangles 5 and 6 cultures at or near the apices where the proportion of either calcium or magnesium was high were the only ones making favorable growth. The conclusion derived from these observations is that, at this concentration, an excess of any one of the nutrient salts may retard root growth, but that excesses of hydrogen, calcium and magnesium ions are the most serious influencing factors. Where favorable growth occurred in solutions with high partial concentration of magnesium, the secondary roots were comparatively well developed, but the root system as a whole was short; while in those cases where good root growth occurred in solutions having a high proportion of calcium, the primary roots were of good length but possessed few and incipient secondary roots.

As has been remarked, during the intervals between changes several of the nutrient solutions of this series formed precipitates. The data of table XXIV show the extent of this condition in the solutions that were employed during the period from the eighteenth to the twenty-first day. The remaining solutions of the series contained slight but insignificant precipitates in all cases.

TABLE XXIV

Relative amounts of precipitate formed in culture solutions during the period from the eighteenth to the twenty-first day of series 7.

Much precipitate	Considerable precipitate	Little precipitate
Culture no.	*Culture no.*	*Culture no.*
T1R1C3	T1R2C3	T1R1C2
T1R1C4	T1R2C5	T1R2C2
T1R1C5	T1R3C3	T1R3C4
T1R1C6	T2R1C3	T2R1C2
T1R2C4	T2R1C4	T2R2C4
	T2R1C5	T3R1C1
	T2R2C3	
	T3R1C3	

Although these precipitates were not examined other than microscopically, there appears little doubt that they consisted chiefly of calcium sulphate. This probability is strengthened by the fact that they occurred in those solutions of the series where calcium nitrate and magnesium sulphate were present in the highest combined proportion to the other salts.

The four pathological phenomena that appeared in the tops of this series will now be described in detail.

(a) Wilting of leaves, followed by death of plants. This phenomenon appeared after three days of growth and increased so rapidly that after only seven days the loss of plants by this injury was severe, twelve cultures being affected and three of these having suffered the total loss of two plants each. A survey of this phenomenon, after 19 days of growth, showed that death of the foliar tissue generally proceeded from the leaf tip downward. On account of the close resemblance of this disturbance, in its early stages, to that form of magnesium injury in which neither appreciable rolling of the leaves nor marked loss of color are involved, this survey may have included the latter phenomenon to a slight extent. The results of this survey are presented in table XXV, where the numbers of dead leaves and of dead plants are set down opposite the corresponding culture numbers.

TABLE XXV

Numbers of leaves and of plants dead on the nineteenth day of series 7.

Culture number	Number of dead leaves	Number of dead plants	Culture number	Number of dead leaves	Number of dead plants	Culture number	Number of dead leaves	Number of dead plants
T1R1C6	9	6	T2R3C3	4	1	T4R1C2	3	1
T1R1C7	6	3	T2R3C4	1	1	T4R1C4	2	1
T1R2C6	1	1	T2R4C1	3	1	T4R2C1	2	1
T1R3C4	2	0	T2R4C2	5	2	T4R2C2	3	1
T1R4C4	4	2	T2R4C3	3	1	T4R2C3	3	1
T1R5C3	7	3	T2R5C1	4	2	T4R3C1	4	2
T1R6C2	8	3	T2R5C2	4	2	T4R3C2	3	1
T1R7C1	2	0	T2R6C1	2	1	T5R1C1	4	1
T2R1C2	2	1	T3R1C1	1	0	T5R1C2	4	2
T2R1C3	6	3	T3R1C5	6	3	T5R1C3	1	0
T2R1C4	1	1	T3R2C2	6	3	T5R2C1	5	2
T2R1C5	2	0	T3R2C3	1	0	T5R2C2	5	2
T2R1C6	8	1	T3R3C1	2	1	T5R3C1	6	3
T2R2C1	1	0	T3R3C3	3	1	T6R1C1	2	1
T2R2C4	6	2	T3R4C1	4	2	T6R1C2	2	1
T2R3C1	2	1	T3R4C2	4	2	T6R2C1	2	0
T2R3C2	2	1	T3R5C1	4	2	T7R1C1	6	2

Comparison of this list of cultures with the triangular diagrams (figure 4) shows that in the three larger triangles this form of injury was most serious along the sides where the proportion of magnesium sulphate was low and especially at the apices representing the maximum proportions of calcium nitrate, while in the other triangles it was generally distributed. This condition appeared first in the larger triangles, where calcium was present in greatest amounts. It seems thus to be causally related to a high proportion of calcium in a concentrated nutrient solution. The lowest osmotic ratio of calcium nitrate to magnesium sulphate where this injury occurred (cultures T2R2C1 and T3R1C1) was 1 : 5 (molecular ratio, 1.0 : 8.3). Here only one leaf was affected.

(b) Yellowing of the basal leaves, followed by death. This injury, which first appeared after 12 days of growth, closely resembled the phenomenon attributed in series 6 to lack of nitrogen. Appearing chiefly on the sides where the proportion of magnesium sulphate was low, it spread rapidly toward the interior of the triangles, becoming, at the later stages of growth, most prominent about the apices of triangles 1 and 2, where the proportion of potassium nitrate was highest. In some cases yellow was replaced by reddish hues, suggesting progressive decomposition of the chlorophyll. After 19 days the following cultures were appreciably affected by this injury, those marked (r) exhibiting prominent reddish coloration: T1R1C7, T1R2C4, T1R3C3, T1R3C5, T1R4C2, T1R4C4, T1R5C1, T1R5C2, T1R5C3, T1R6C1, T1R7C1, T2R1C6(r), T2R2C5(r), T2R3C4, T2R4C2, T2R4C3, T2R5C1, T2R5C2, T2R6C1, T3R3C1, T3R3C2, T3R3C3, T3R4C1, T3R4C2(r), T3R5C1(r), T4R1C3, T4R1C4, T4R2C2, T4R2C3, T4R3C2, T4R4C1, T5R2C2, T5R3C1, T6R1C2, T6R2C1, DW(r).

The coloring here recorded for the control plants (DW) was also observed in the control cultures of other series, and appeared to be one of the changes usually accompanying maturity and death, consequent to the exhaustion of a limited supply of nutrient compounds. Apparently this phenomenon in the other cultures of series 7 was also the forerunner of premature death, and probably resulted from unfavorable proportions of the nutrient salts. There may be some significance in the fact that the affected cultures were confined largely to the regions of the triangular diagrams corresponding to media with the lowest proportions of magnesium sulphate; but this injury was not clearly connected with poor root development nor with any other visible disturbance.

(c) Foliar striping, due to bleaching of the tissue between the veins. The yellowing of leaf tissue between the veins was observed in its early stages after 12 days of growth. After 19 days it was conspicuous in the the following cultures: T1R2C4, T1R3C4, T1R3C5, T1R4C4, T1R5C3, T1R7C1, T2R3C3, T2R4C2, T2R4C3, T2R5C1, T2R5C2, T2R6C1, T3R3C2, T3R4C1, T3R4C2, T3R5C1 T4R2C2, T4R2C3, T4R3C1, T4R3C2, T4R4C1, T5R1C2, T5R2C1, T5R2C2, T5R3C1, T6R1C1, T6R1C2. These cultures are distributed in triangles 1 and 2, over regions near the apices representing high content of potassium nitrate and along the sides where the proportion of magnesium sulphate was low, although the latter distribution is irregular in triangle 1. In the remaining triangles the affected cultures are quite sharply confined to a rhombic area, of which the triangle apex representing high partial concentration of potassium nitrate formed one of the acute angles, and the adjacent cultures containing calcium nitrate and magnesium sulphate in the osmotic proportions 1:2 and 2:1 formed the obtuse angles. In cultures having the lower proportions of mono-potassium phosphate, foliar striping appeared with low proportions of magnesium sulphate but not with the highest proportion of calcium nitrate. It may thus be seen that its occurrence was accompanied by high content of phosphate, a maximum content of potassium nitrate, and approximately equal contents of calcium nitrate and magnesium sulphate. This disturbance appears to have occurred throughout a considerable range of proportions of the chemical elements and ions in the nutrient media. It probably resulted from the combined effects of the high total concentration of the solutions and certain unfavorable proportions of the nutrient salts.

(d) Magnesium injury. The characteristic rolling of the wheat leaves due to high partial concentration of magnesium sulphate in the medium, as described for series 5, appeared after seven days in series 7. In triangles 5, 6 and 7 (see figure 4), the injured leaves rolled only loosely and at first without discoloration; hence, as previously remarked, it was difficult to distinguish this injury in its early stages from the phenomena of wilting (a, above). In later stages, however, the two forms of disturbance were quite clearly differentiated. Table XXVI presents the results of surveys of the progress of this injury, taken at intervals of from three to five days. In each survey the number of leaves per culture severely injured and also the number slightly injured were recorded. All of the culture numbers are listed, even though no injury is to be recorded.

TABLE XXVI

Survey of magnesium injury in series 7

Number of days from beginning of culture.	7		10		14		19		22	
Culture number	Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T1R1C1	..	..	..	2	1	1	1	1	3	1
C2	..	..	..	..	..	..	..	1	..	1
C3	..	1	..	1	..	1	..	1	1	.
C4	..	..	..	1	..	2	..	2	..	2
C5	..	..	..	..	..	..	..	..	1	1
C6	..	..	..	1	..	2	..	2	5	
C7	..	..	..	1	..	1	..	1	2	
R2C1	..	..	..	1	..	1	2	1	3	2
C2	..	..	..	..	..	..	..	..	..	.
C3	..	..	..	1	..	1	..	1	..	1
C4	..	..	..	1	..	1	..	1	1	1
C5	..	..	..	5	..	5	..	5	..	5
C6	..	..	..	1	..	1	..	2	5	
R3C1	..	..	..	..	..	2	..	2	4	1
C2	..	..	..	..	..	..	..	..	..	.
C3	..	..	..	1	..	1	..	1	..	3
C4	..	..	..	..	..	..	..	..	..	
C5	..	1	..	3	..	3	..	3	..	3

TABLE XXVI—Continued

Number of days from beginning of culture.	7		10		14		19		22	
Culture number	Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T1R4C1..........	..	..	..	2	..	2	..	2	3	..
C2..........	..	..	..	..	..	..	..	..	..	1
C3..........	..	..	..	1	..	1	..	1	..	1
C4..........	1	..	1	..	1	..	1	..	1	1
R5C1..........	..	..	..	..	..	1	..	3	5	..
C2..........	..	..	..	..	..	..	..	..	..	2
C3..........	..	..	1	..	1	..	1	..	1	..
R6C1..........	..	..	..	..	..	..	..	..	..	..
C2..........	..	..	..	..	..	..	..	..	..	..
R7C1..........	..	1	..	1	..	2	..	2	..	3
T2R1C1..........	..	..	..	1	..	2	..	3	4	2
C2..........	..	..	..	..	..	..	..	..	..	..
C3..........	..	..	..	..	..	..	..	..	3	1
C4..........	..	..	..	1	..	1	..	1	3	..
C5..........	..	..	..	1	..	1	..	1	2	2
C6..........	..	..	..	1	..	1	..	1	..	1
R2C1..........	..	..	..	..	..	..	..	1	4	1
C2..........	..	..	..	..	..	..	..	..	..	..
C3..........	..	..	..	1	..	1	..	1	..	1
C4..........	..	..	..	1	..	1	..	1	1	1
C5..........	..	..	..	1	1	1	1	1	1	1
R3C1..........	..	..	..	..	..	..	1	..	3	1
C2..........	..	..	..	..	..	..	..	..	..	..
C3..........	..	..	..	1	..	1	..	1	1	..
C4..........	..	..	..	..	..	..	..	..	1	1
R4C1..........	..	..	..	..	..	..	2	..	2	..
C2..........	..	..	..	..	..	..	..	..	..	1
C3..........	..	..	1	..	1	..	1	..	2	1
R5C1..........	..	..	..	..	..	..	..	..	2	..
C2..........	..	..	..	..	..	..	..	..	..	..
R6C1..........	..	..	..	..	..	..	..	..	..	3
T3R1C1..........	..	..	..	2	1	1	2	1	5	1
C2..........	..	..	..	..	..	..	..	..	3	..
C3..........	..	..	..	1	..	2	..	2	..	2
C4..........	..	..	..	..	..	..	..	..	..	..
C5..........	..	..	1	1	1	1	1	1	1	1

TABLE XXVI—Continued

Number of days from beginning of culture.	7		10		14		19		22	
Culture number	Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury		Leaves showing injury	
	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight	Severe	Slight
T3R2C1	..	..	..	..	..	1	..	3	6	..
C2	..	..	..	1	..	1	..	1	2	..
C3	..	..	..	1	..	1	..	1	3	1
C4	..	..	..	..	..	1	..	..	2	3
R3C1	..	..	..	2	..	2	..	2	5	..
C2	..	..	..	..	..	..	..	..	5	3
C3	..	..	..	1	..	1	..	1	2	..
R4C1	..	..	..	..	..	..	1	1	6	1
C2	..	..	..	..	..	..	..	..	..	1
R5C1	..	..	..	1	..	2	..	2	2	..
T4R1C1	..	..	..	1	..	1	..	3	5	1
C2	..	..	..	..	..	..	..	..	5	2
C3	..	..	..	..	..	..	..	..	4	2
C4	..	..	..	..	..	1	..	1	1	..
R2C1	..	..	..	..	..	..	..	..	2	..
C2	..	..	..	..	1	..	1	..	5	2
C3	..	1	1	2	1	2	1	2	6	..
R3C1	..	..	..	1	..	1	..	1	2	..
C2	..	..	1	1	1	1	1	1	2	4
T4R4C1	..	..	..	1	..	1	..	2	4	1
T5R1C1	..	1	..	1	..	1	4	..	8	..
C2	..	1	..	1	..	1	..	1	4	..
C3	..	..	..	1	..	1	2	2	5	2
R2C1	..	..	1	1	1	1	3	..	5	..
C2	..	..	..	1	..	1	1	..	2	2
R3C1	..	..	1	1	1	1	1	1	1	1
T6R1C1	..	..	2	..	2	1	2	1	5	1
C2	..	..	..	2	..	2	..	2	3	2
R2C1	..	..	..	2	..	2	2	1	6	1
T7R1C1	..	1	1	2	1	3	3	1	6	1

The most prominent feature of the magnesium injury in this series, as shown by the data of table XXVI, is the tardiness of its appearance in the majority of the cultures. On the last date indicated in the table (twenty-second day) there was a marked increase in this injury, as a result of its general appearance in the young, third and fourth leaves, which coiled in the manner mentioned in connection with series 5. Comparison with the corresponding surveys of the latter series shows that, while a much smaller number of leaves per culture were affected, the injury was somewhat more widely spread in the present series, in which 75 out of the 84 solution cultures developed this disturbance. Thus, while series 5 contained only 8 injured cultures not also occurring in series 7 (T1R2C2, T1R3C2, T1R3C4, T1R6C1, T1R6C2, T2R1C2, T2R2C2 and T2R3C2), series 7 contained sixteen injured cultures not occurring in series 5 (T1R1C6, T1R2C5, T1R2C6, T1R3C5, T2R1C5, T2R1C6, T2R2C4, T2R2C5, T2R3C4, T2R4C3, T3R1C5, T3R2C4, T3R3C3, T4R1C4, T4R2C3 and T5R1C3). The cultures injured in series 5 but not injured in series 7 are those characterized by having two-tenths of the total osmotic pressure of the medium due to calcium nitrate, with various proportions of magnesium sulphate. The cultures injured in series 7 but not injured in series 5 lie, in the triangular diagrams, at the apices highest in partial pressure of calcium nitrate and in the adjacent areas, particularly along the triangle side representing the lowest content of magnesium sulphate.

Another striking feature of the magnesium injury in this series is its progressive increase from the larger to the smaller triangles, that is, from the lower to the higher proportions of mono-potassium phosphate. Thus, if the affected leaves are calculated to an average number per culture for the several triangles, the following results are obtained:

Triangle Number.		1	2	3	4	5	6	7
Average number of leaves per culture showing. .	Pronounced injury......	1.5	1.6	3.0	3.6	4.1	4.7	6.0
	Incipient injury........	1.3	1.0	1.0	1.2	0.8	1.3	1.0

It will thus be seen that, while the number of leaves in the early stages of injury remains rather constant throughout the seven triangles, the serious injury increases rapidly as the partial concentration of potassium phosphate in the solution increases. This emphasizes the inter-relation of all four salts in determining the conditions that control the harmful effect of magnesium. No doubt the acidity due to mono-potassium phosphate influenced this effect in series 7.

If the data for the twenty-second day of series 7 (table XXVI) are analyzed after a manner similar to that employed in the corresponding case of series 5, any relations here existing between the magnitude of the osmotic ratio of magnesium sulphate to calcium nitrate and the occurrence of the pathological disturbance in question should become clear. In the present case all cultures showing injury of five or more leaves have been grouped into one category, those with only one leaf injured form a second group, and those with no injury apparent comprise a third. These groups, together with the osmotic ratios in question, are set forth in table XXVII.

TABLE XXVII

Relation of leaf injury to the value of the osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$, in series 7.

Five or more leaves severely injured		Only one leaf injured		No injury	
Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$	Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$	Culture number	Osmotic ratio, $\frac{MgSO_4}{Ca(NO_3)_2}$
T1R1C6	0.33	T1R1C2	3.00	T1R2C2	2.50
R2C6	0.17	C3	1.67	R3C2	2.00
R5C1	3.00	R2C3	1.33	C4	0.50
T3R1C1	5.00	R4C2	1.50	R6C1	2.00
R2C1	4.00	C3	0.67	C2	0.50
R3C1	3.00	R5C3	0.33	T2R1C2	2.50
C2	1.00	T2R1C6	0.17	R2C2	2.00
R4C1	2.00	R2C3	1.00	R3C2	1.50
T4R1C1	4.00	R3C3	0.67	R5C2	0.50
C2	1.50	R4C2	1.00	T3R1C4	0.50
R2C2	1.00	T3R4C2	0.50		
C3	0.33	T4R1C4	0.25		
T5R1C1	3.00				
C3	0.33				
R2C1	2.00				
T6R1C1	2.00				
R2C1	1.00				
T7R1C1	1.00				

A glance at table XXVII shows clearly that there is no such simple relation here brought out as was found by similar treatment of the corresponding data of series 5; there is obviously no generalization to be obtained by attempting to study here the maximum and minimum ratios of magnesium sulphate to calcium nitrate. It is clearly evident, however, that the antagonistic action between magnesium and calcium is greatly influenced by the total concentration of the medium when the osmotic proportions of the various salts are allowed to remain constant. A high proportion of magnesium sulphate to the remaining salts produced more generally prevalent, but less serious, injury with the solutions of series 7 (2.0 per cent. total concentration, with osmotic pressure of 8.15 atmospheres) than with the solutions of series 5 (0.6 per cent. total concentration, with osmotic pressure of 2.50 atmospheres).

Photographs of the cultures of triangle 2 and of selected cultures of triangle 1, series 7, taken on the twenty-third day of the period of this series and corresponding to the similar photographs already presented for series 5 and 6, are reproduced as figures 11 and 12. They illustrate the general range of appearance of the plants at this time, just before harvest.

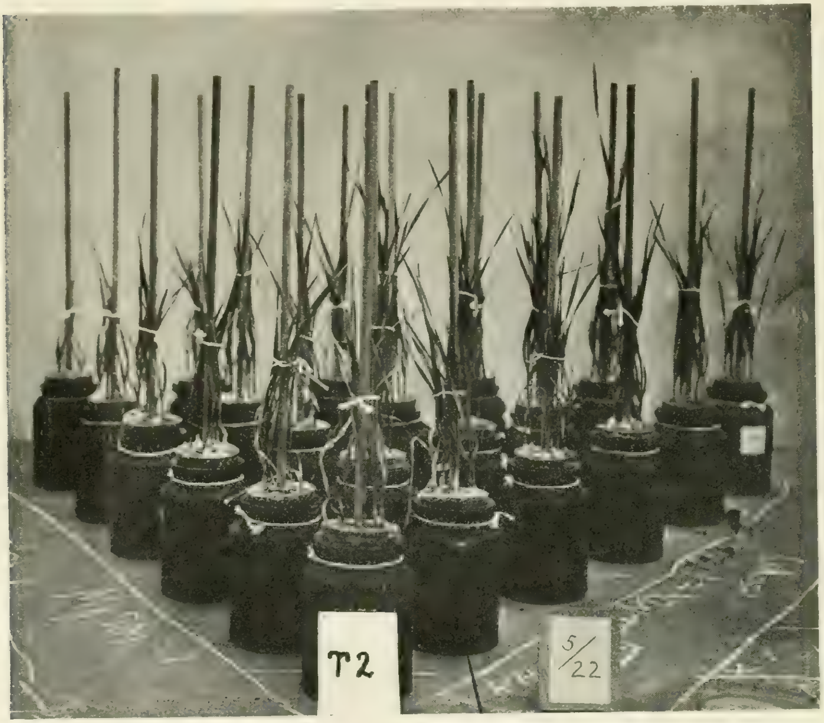

Figure 11. Triangle 2 of series 7, on twenty-third day. The culture nearest the observer has the highest content of KNO_3, that at the extreme right has the highest content of $MgSO_4$. (See also figure 4 for the arrangement.) Poorest growth shown at extreme left, highest content of $Ca(NO_3)_2$. Poor growth also shown at extreme right.

Yields of Series 7. After 24 days of growth the cultures of series 7 were harvested in the usual manner. The six control plants in distilled water gave a yield of 0.1596 g. dry weight of tops and 0.1062 g. dry weight of roots. The yields of the various solution cultures, relative to the control as unity, have been plotted upon the triangular diagrams of figure 13, in conformity with the methods employed for similar data of series 5 and 6. On account of the unfavorably high concentration of the nutrient solutions of this series, no correction has been made for weak or dead plants.

The greatest relative dry weight of tops in this series (2.92) occurred in culture T1R4C2, in which the osmotic proportions of nutrient salts were $KH_2PO_4:MgSO_4:Ca(NO_3)_2:KNO_3 = 0.25:0.75:0.50:1.00$. The smallest yields of tops (0.81) was obtained from culture T2R1C6, which places the value of the best yield at 3.6 times that of the poorest. This range is 1.7 times as great as that derived from either series 5 or 6 (2.1), which agreed in this respect. Since culture T1R5C1, closely adjacent to the optimum T1R4C2, produced a dry weight (2.78) nearly equal to that of the latter culture (with osmotic proportions of the salts, in the same order as above, 0.2 : 0.6 : 0.2 : 1.0), these two cultures may be averaged for the optimum of the series. This optimal average is 2.85, with the osmotic proportions of salts, 2.2 :6.7 :3.3 :1.0 (molecular proportions, 0.23 :0.81 :0.25 :1.00). In series 7 was obtained, therefore, a relative yield of tops 53.23 per cent. greater than that from Knop's solution (1.86, table XIV, p. 185), in which the osmotic proportions of salts were 0.7 : 0.7 : 3.1 : 1.0 (molecular proportions, 0.76 : 0.85 : 2.53 : 1.00). The derived optimal solution of series 7 contained only 0.80 as much KH_2PO_4, 0.25 as much $Ca(NO_3)_2$, but 2.5 times as much $MgSO_4$ and 2.7 times as much KNO_3, on the molecular basis, as did the Knop's solution of series 4. The most extensive changes from Knop's formula here involved were decreases in calcium and nitrate and increases in magnesium, potassium and sulphate. From these changes it is strongly suggested that the derived optimum nutrient solution of series 7

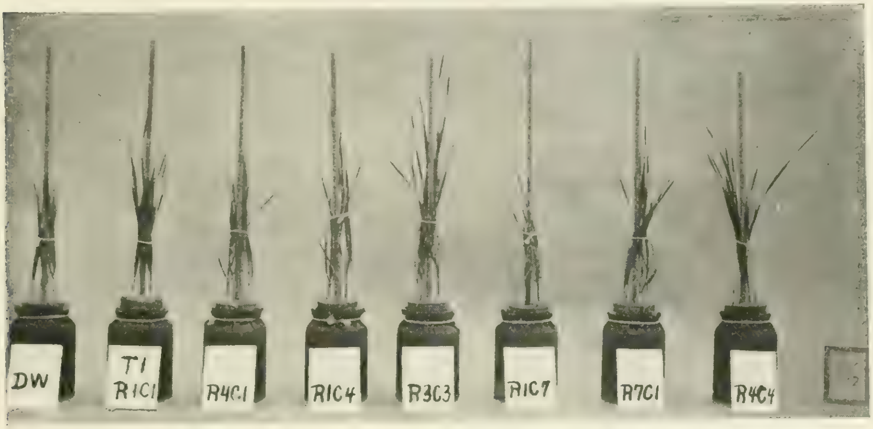

Figure 12. Selected cultures from triangle 1, series 7 on twenty-third day.

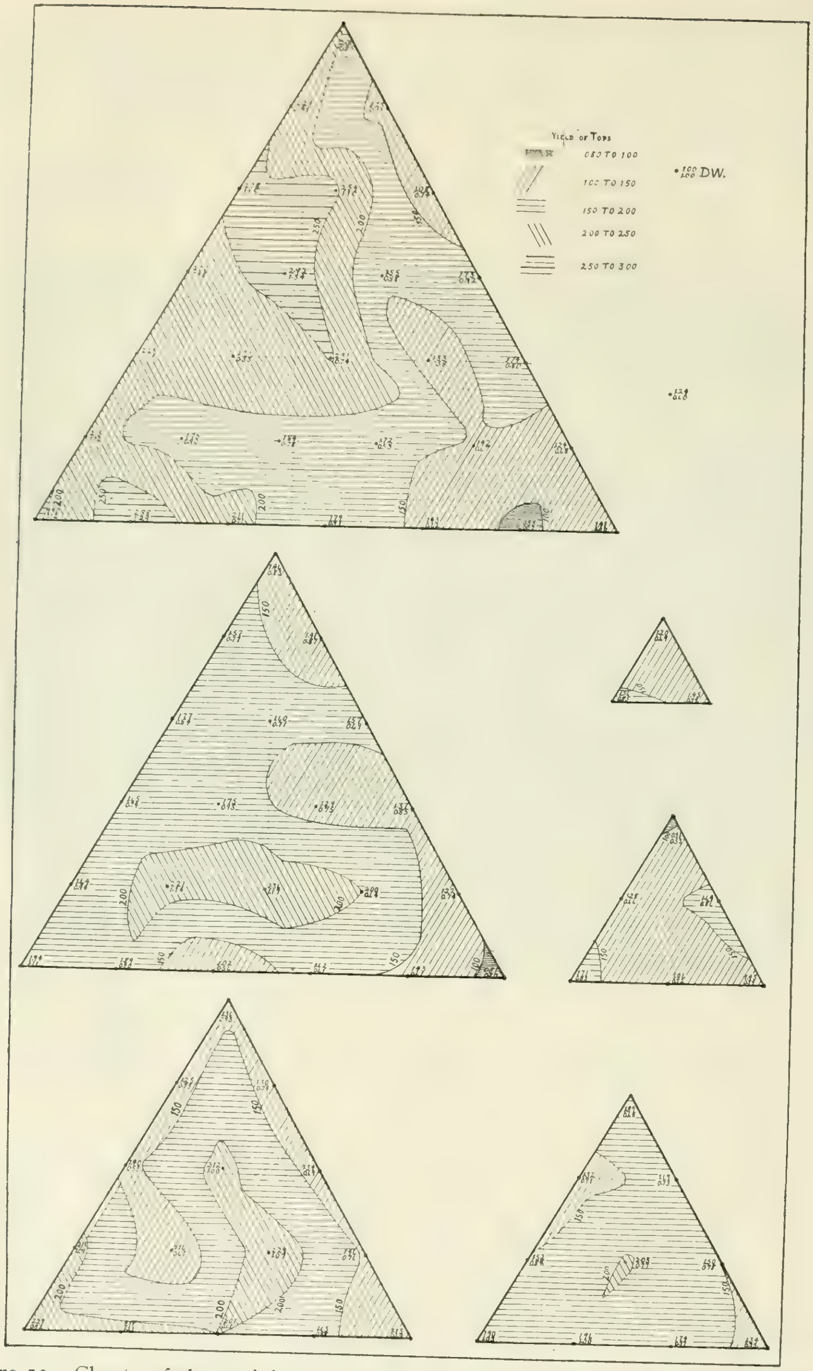

Figure 13. Charts of dry weights of tops and roots of cultures of wheat from series 7, six plants per culture, grown twenty-four days in nutrient solutions containing the salts of Knop's formula in varying proportions but equal in osmotic pressure. Total concentration, 7.63 atmospheres of osmotic pressure, or 2.0 per cent. The upper number in each position represents weight of tops, the lower that of roots. The different areas of the triangles, according to weight of tops, are shaded differently. (See also figure 4, for culture numbers and numbers of triangles.)

(with its high total concentration) derives its greater efficiency to produce tops chiefly from a high ratio of magnesium and potassium, on the one hand, to calcium on the other.

The maximum yield of dry roots, relative to the control, (1.34) was produced by culture T1R4C2, while the minimum relative yield (0.33) was produced by culture T1R3C2. The former yield was, therefore, 4.1 times the latter, which gives to the range of the relative yields of roots in this series a somewhat higher magnitude than that obtained for the range of tops. This range of the relative dry weights of roots in series 7 is about twice as great as the corresponding range in either series 5 or 6. The osmotic proportions of salts in the best and poorest culture solutions (on the basis of dry weight of roots) were $KH_2PO_4 : MgSO_4 : Ca(NO_3)_2 : KNO_3 = 0.25 : 0.75 : 0.50 : 1.00$ and $0.3 : 1.3 : 0.7 : 1.0$, respectively. Since culture T1R1C2, having a relative dry weight of roots of 1.29 (with the osmotic proportions of salts, 1 : 6 : 2 : 1) closely approached the efficiency (1.34) of the best solution (T1R4C2), these two yields may be averaged to give 1.32 as the derived optimum root yield of this series. Averaging the two series of osmotic proportions of salts gives the osmotic proportions, 0.4 : 1.8 : 0.8 : 1.0 (molecular proportions, 0.65 : 4.13 : 0.92 : 1.00), which may be taken as the derived proportions of salts for the optimum solution. The relative yield of roots in the 2.0 per cent. Knop's solution of series 4 was 0.9, which gives, to the derived optimum of series 7, 46.7 per cent. greater efficiency than was shown by Knop's solution. This superiority over Knop's solution agrees well with that observed in the case of tops (53.23 per cent.).

This efficiency of the derived optimum of series 7 is about one-half greater than that of the corresponding optimum of series 5 and three times as great as that of the optimum of series 6, and accords with the relatively high efficiency of this series in respect to dry weight of tops. The average yield of roots from cultures T1R4C2 and T1R5C1, which produced the highest dry weight of tops, was 1.25, or 38.9 per cent. greater than that obtained from the corresponding Knop's solution in series 4.

In contrast to the two preceding series there appears to be a somewhat vague but nevertheless observable relation between the dry weights of roots and of tops in series 7, especially in the cultures producing the greatest yields. It may be observed, for example, that the greatest weights of tops and of roots occurred in the same culture (T1R4C2) and also that cultures T1R1C2 and T1R5C1, with relative yields of tops of 2.88 and 2.78, respectively, produced yields of roots of 1.29 and 1.15, respectively. On the other hand, the culture (T1R3C2) giving the lowest yield of roots (0.33) produced a comparatively good yield of tops (2.36) while the next lowest yield of roots (0.53) was produced by each of three cultures (T1R2C4, T3R3C1 and T5R3C1) which produced dry weights of tops of 1.72, 1.40 and 0.91, respectively. It is thus apparent that correlation of growth between tops and roots does not hold, even in this series, to any great extent.

Transpiration rates for selected cultures in series 5, 6 and 7.

Transpiration data for selected cultures of series 7 are presented in table XXVIII, together with corresponding data from the two preceding series. The data of series 5 refer to a 24.5-hour period on the twenty-second and twenty-third days of growth, those of series 6 refer to a 28.0-hour period on corresponding days, and those of series 7, on account of the small size of the plants and the high humidity of the atmosphere at the time of observation in this regard, were extended over a period of 56.5 hours, from the twentieth to the twenty-third day of growth. The cultures employed were so selected as to be evenly distributed throughout each series, according to the arrangement of the cultures on the triangular diagrams. The water losses from the various cultures have been reduced to the basis of the loss from the control in distilled water, the latter loss considered as unity, thus giving relative water losses,[90] which are presented in the table. For purposes of comparison the ratios of relative water losses to relative dry weights of tops are also included in this table.

According to the data of table XXVIII the greatest water loss in series 5 (culture T4R1C4) was 2.71 times the least (culture T2R3C1); in series 6 the greatest water loss (culture T1R3C3) was 2.12 times the least (culture T4R1C1) and in series 7 the greatest water loss (culture T1R5C2) was 5.85 times the least (culture T4R4C1). The narrowest range of transpiration therefore occurred in series 6 which was practically free from injury to the plants, and the widest range occurred in series 7, where the greatest injury occurred. If we group together the six highest and six lowest cultures of series 5, with regard to the ratio of transpirational water loss to the dry weight of tops, it is found that the cultures of lowest ratio (T1R4C4, T1R1C7, T1R7C1, T2R1C4, T3R3C1 and T7R1C1) possessed a total of 33 leaves affected by magnesium injury, while those of the highest ratio (T1R2C2, T2R6C1, T4R1C4, T5R1C2, T5R3C1, and T6R1C1) possessed 39 leaves similarly injured. The same treatment applied to series 7 gives for the group of cultures having lowest ratios (T1R7C1, T2R3C4, T4R4C1, T5R1C3, T7R1C1 and the average of the equally transpiring cultures T1R1C4, T3R3C2 and T6R1C1) a total of 29 leaves injured by magnesium, while the group of cultures with highest ratios (T1R1C7, T1R2C2, T2R3C1, T2R4C2, T3R1C5 and the average of the equally transpiring cultures T1R4C1 and T1R4C4) possessed only 12 injured

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[90] The term *relative water loss* is here used instead of *relative transpiration* to avoid an ambiguity which has recently developed in the literature of transpiration; the latter expression is used in two entirely different senses. One meaning is *water loss in terms of the loss from a standard plant or culture as basis of comparison*, as in the present instance, and the other is *the ratio of the water loss from a plant or culture to that from some standard physical evaporating surface*, as of an atmometer. Livingston [06] introduced the expression in the latter sense and, in the following year [07] employed the same term extensively in the other sense. The relative transpiration ratio (as first used) has become so important in discussions of the water relations of organisms that it seems desirable to reserve the term here considered for that ratio and to employ another for the less specific meaning.

TABLE XXVIII

Relative transpirational water losses for selected cultures, series 5, 6 and 7, for a period just preceding harvest, together with the corresponding ratios of relative water loss to relative dry weight of tops.

Culture number	Series 5 (Water loss from standard atmometer, 12.13 cc. in 24.5 hrs.)		Series 6 (Water loss from standard atmometer, 12.05 cc. in 28 hrs.)		Series 7 (Water loss from standard atmometer, 22.48 cc. in 56.5 hrs.)	
	Relative water loss	Ratio of relative water loss to relative dry weight of tops	Relative water loss	Ratio of relative water loss to relative dry weight of tops	Relative water loss	Ratio of relative water loss to relative dry weight of tops
DW	1.00 (1.70)*	1.00	1.00 (1.65)*	1.00	1.00 (7.30)*	1.00
T1R1C1	4.73	1.36	8.70	2.74	1.29	0.68
C4	6.62	1.24	12.15	3.58	0.80	0.45
C7	4.64	1.04	14.35	3.55	1.16	0.82
R2C2	5.47	1.41	13.40	4.24	1.81	1.03
C5	6.62	1.27	11.80	4.10	0.76	0.53
R3C3	6.91	1.30	19.25**	3.80	1.38	0.55
R4C1	4.32	1.18	11.45	3.14	1.60	0.80
C4	4.06	0.85	16.05	3.48	1.38	0.80
R5C2	5.91	1.20	13.85	3.51	1.93	0.76
R7C1	4.94	1.07	14.00	3.36	0.79	0.39
T2R1C1	4.23	1.32	9.15	3.36	1.39	0.72
C4	6.35	1.09	13.40	3.54	1.02	0.63
C6	6.50	1.29	15.15	3.56	0.58	0.72
R2C3	6.18	1.18	13.10	3.34	1.68	0.71
R3C1	3.20	1.15	9.88	3.66	1.37	0.83
C4	....	....	16.85**	3.95	0.40	0.29
R4C2	6.06	1.34	14.95	3.78	1.42	0.89
R6C1	6.68	1.55	10.50	3.12	0.80	0.55
T3R1C1	4.70	1.15	8.30	2.96	1.36	0.59
C3	6.56	1.31	11.05	3.40	1.21	0.60
C5	6.44	1.23	11.25	3.35	0.96	0.85
R2C3	....	....	12.35	3.46	1.70	0.74
R3C1	3.91	1.11	10.50	3.30	1.10	0.79
C2	5.91	1.18	11.60	3 50	0.96	0.45
C3	5.14	1.14	12.25	3.55	0.90	0.67
R5C1	6.32	1.29	12.80	3.56	0.80	0.59
T4R1C1	4.61	1.19	8.12	3.05	1.18	0.62
C4	8.68	1.63	12.25	3.28	0.68	0.81
R2C2	6.56	1.26	10.05	3.40	1.15	0.57
R4C1	5.58	1.38	10.95	3.29	0.33	0.17

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*The actual transpirational loss from the control culture, which forms the basis for the entire series, is here indicated in parenthesis. From this value and the relative value for any other culture may readily be calculated the actual water loss for that culture.

**Calculated from data for five plants.

TABLE XXVIII—Continued

Culture number	Series 5 (Water loss from standard atmometer, 12.13 cc.)		Series 6 (Water loss from standard atmometer, 12.05 cc.)		Series 7 (Water loss from standard atmometer, 22.48 cc.)	
	Relative water loss	Ratio of relative water loss to relative dry weight of tops	Relative water loss	Ratio of relative water loss to relative dry weight of tops	Relative water loss	Ratio of relative water loss to relative dry weight of tops
T5R1C1	5.11	1.14	8.88	3.50	1.15	0.67
C2	6.53	1.45	9.58	3.55	0.80	0.75
C3	5.94	1.22	11.95	3.44	0.47	0.33
R3C1	6.79	1.49	10.50	3.76	0.59	0.65
T6R1C1	5.35	1.47	8.15	3.28	0.70	0.45
C2	7.32	1.40	9.70	3.39	0.96	0.66
R2C1	6.29	1.38	9.27	3.38	0.91	0.76
T7R1C1	6.08	1.12	8.72	3.15	0.36	0.29

leaves. In series 5, therefore, no marked correlation is apparent between magnesium injury and water loss per unit of dry weight of tops, but in series 7 it is plainly evident that greatly decreased ratios of water loss to yield of tops accompanied excessive magnesium injury. Apparently the transpiring power of the leaves (depending upon area, condition of the epidermis, etc.), was reduced upon the injured leaves in the latter series, where comparatively little growth occurred, but with the larger plants and greater leaf area characteristic of the previous series magnesium injury had slight, if any, influence upon the foliar transpiring power. In terms of the ratio of relative water loss to relative dry weight series 5 ranged from 1.0 to 1.9, series 6 from 1.0 to 1.5 and series 7 from 1.0 to 6.0. The foliar transpiring power per unit of dry weight of tops was therefore depressed slightly in series 5 and seriously in series 7, as compared with series 6, which was free from magnesium injury. A graphical comparison of this transpiring power per unit of weight of tops, for cultures listed in table XXVIII, is presented in figure 14, where the abscissæ are arbitrarily taken to represent the different cultures and the ordinates represent the ratios under discussion. Since all data are relative to the control culture of the corresponding series, considered as unity, all three graphs pass through a common point (0, 1), and are comparable throughout. The uniformly high ratios exhibited by the cultures of series 6, as compared to those shown by the cultures of series 5 and 7 are clearly brought out; as is also the fact that the ratios are uniformly lower for series 7 than for series 5.

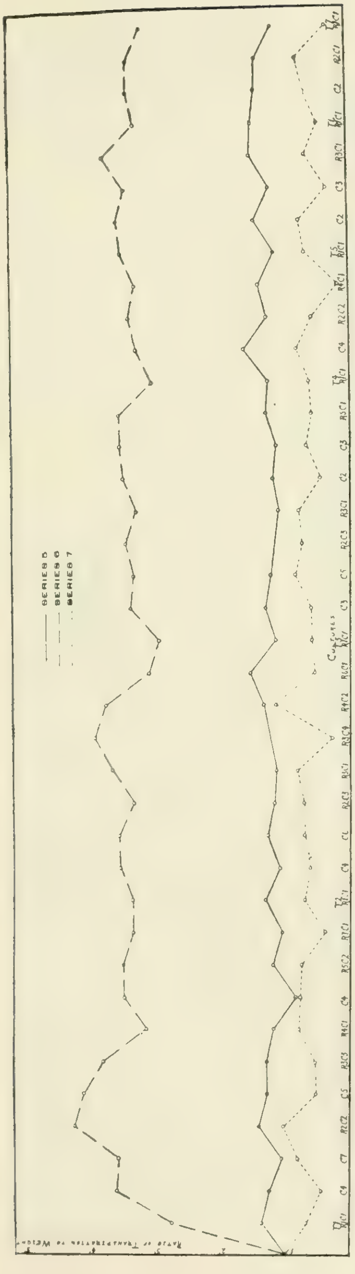

Figure 14. Graphs showing relative ratios of water loss to dry weight of tops, for series 5, 6 and 7.

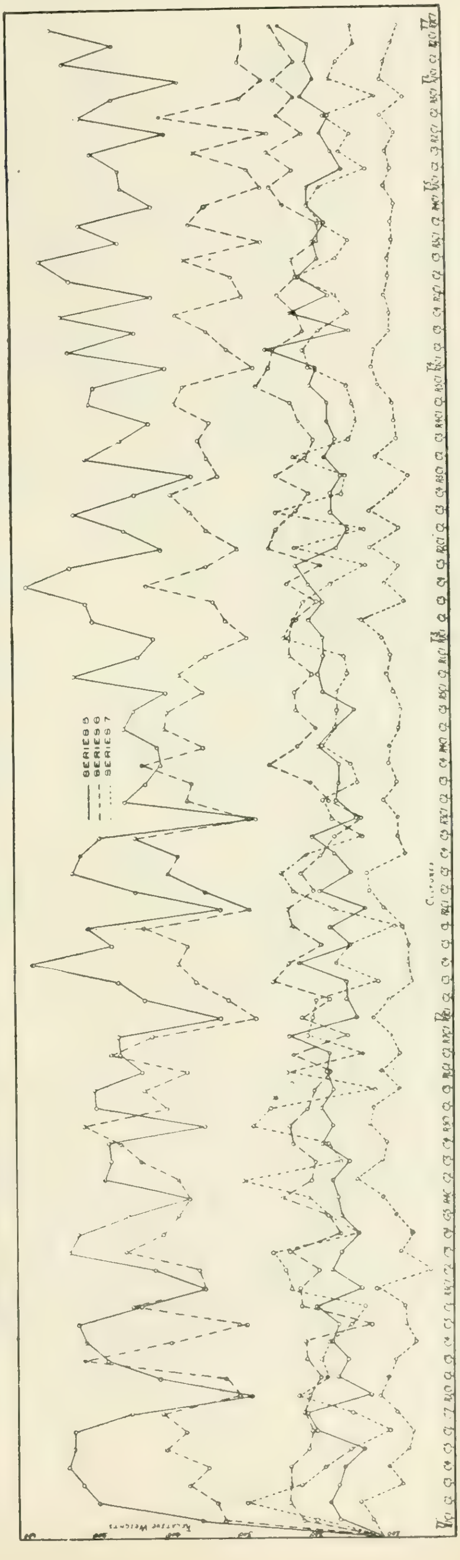

Figure 15. Graphs showing relative dry weights of tops and of roots from series 5, 6 and 7. The upper graph for each series represents tops; the lower, roots.

SUMMARY OF PART III.

Methods for germinating wheat seeds and for mounting the seedlings have been described, which differ in certain details from those heretofore employed, the most important modification here introduced being the two-piece cork stopper used in the culture jars.

The osmotic pressure of a nutrient solution composed of the four salts used in Knop's solution has been approximately calculated from the data of electrolytic dissociation for the component salts.

Preliminary cultures of wheat in two forms of Knop's nutrient solution, one with di- and the other with mono-potassium phosphate, produced, in 24 days, approximately equal yields of dry matter, throughout a range of total concentration of the medium of from 0.05 to 0.80 per cent. (from 0.22 to 3.20 atmospheres of osmotic pressure). In these cultures mono-potassium phosphate proved to be preferable to the di-potassium salt, in the composition of this nutrient solution; the medium prepared with the former salt produced, under otherwise similar conditions, 17.8 per cent. better growth of tops and 17.5 per cent. better growth of roots, according to the criterion of dry weights, than did the similar solution prepared with di-potassium phosphate.

The effect, upon the growth of wheat seedlings for 24 days, of eighty-four different variations in the relative proportions in which the salts entered into the composition of the nutrient solution, was studied for three different total concentrations of the latter. In each of these three series (numbered 5, 6 and 7) the total osmotic concentration of the medium was approximately the same throughout the eighty-four different cultures, but every culture differed from every other one in the manner in which this total osmotic concentration was distributed among the four partial concentrations produced by the different component salts, respectively. The partial osmotic pressure due to each salt was varied by increments equal to one-tenth of the total pressure of the solution, and all such variations which are possible (eighty-four) were included in each of these three series. The four salts employed were mono-potassium phosphate, magnesium sulphate, calcium nitrate and potassium nitrate. The three series had total concentrations of 0.6 per cent. (2.50 atmospheres osmotic pressure), 0.01 per cent. (0.05 atmospheres of pressure), and 2.0 per cent. (8.15 atmospheres of pressure), the first concentration being taken to represent the optimum, in this respect, for the best growth of the plants. With each series was included a control culture in distilled water. The evaporating power of the air in the greenhouse where these series of cultures were carried out was recorded from readings of a Livingston standard atmometer operated throughout all these series, and the comparative rates of transpiration of selected cultures from each series were determined for a short period just preceding harvest.

The molecular proportions of the four salts (that for potassium nitrate being taken as unity) in the culture solutions which gave the highest dry weights of tops and roots, together with the magnitudes of these yields,

TABLE XXIX

Summary of the molecular proportions of salts in the optimum culture solutions of series 5, 6 and 7, the dry weight yields therefrom, and the corresponding yields from Knop's solution, series 4.

Culture solution	Approximate total osmotic pressure	Total salt concentration	Volume-molecular partial concentrations of the three other salts, compared to concentration of KNO_3 as unity			Dry weight (tops or roots)*		
						Relative to control as unity	Relative to corresponding yield from	
			KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$		Knop's solution (series 2 and 4) as 100	Optimum of series 5 as 100
	Atmospheres	*Per cent.*	*m*	*m*	*m*			
Knop's solution.	2.50 0.05 8.15	0.60 0.01 2.00	0.76	0.85	2.53	....	......	.....
Series 5, optimum for tops*......	**2.50**	**0.60**	**2.66**	**2.97**	**2.95**	**5.84**	**111.00**	...
Series 5, optimum for roots.......	2.50	0.60	1.07	0.30	0.18	2.25	129.10	...
Series 6, optimum for tops.......	**0.05**	**0.01**	**0.34**	**2.00**	**1.00**	**5.10**	**138.96**	**88.54**
Series 6, optimum for roots.......	0.05	0.01	0.41	0.20	0.13	2.63	115.35	83.37
Series 7, optimum for tops.......	**8.15**	**2.00**	**0.23**	**0.81**	**0.25**	**2.85**	**153.23**	**50.50**
Series 7, optimum for roots.......	8.15	2.00	0.41	2.20	0.59	1.32	146.67	59.67

*Data for tops are in full-face type, to facilitate comparisons.

relative to the yield of the distilled water control, are summarized for each series in table XXIX. In the same table are also given corresponding yields from cultures in Knop's solution, and data for comparing each optimum yield with that from the latter solution as well as with the optimum yield obtained in series 5. The absolute molecular concentrations of the four salts in each of these solutions are set forth in table XXX.

The yields both of tops and of roots varied least in series 5 and most in series 7. The root yields showed, in series 6 and 7, greater variation than did the top yields, while in series 5 the yields of tops showed somewhat greater variation than did those of roots. These points are brought out by the following ratios of the greatest to the smallest yields: series 5, tops, 2.10; roots, 1.98; series 6, tops, 2.12; roots, 2.29; series 7, tops, 3.60; roots, 4.10. The greatest yield of tops and also that of roots, in each of the three series, was superior to that from the corresponding Knop's solution, this difference being least in the case of the optimum total concentration (series 5) and greatest in case of the supra-optimal concentration (series 7).

TABLE XXX

Absolute volume-molecular concentrations of the four salts in solutions referred to in table XXIX (series 5, 6 and 7).

Solution	Total concentration	Volume-molecular concentration			
		KH_2PO_4	$MgSO_4$	$Ca(NO_3)_2$	KNO_3
	per cent.	*g. mol. per l.*	*g. mol. per l.*	*g. mol. per l.*	*g. mol. per l.*
Series 5, optimum for tops	0.60	.0130	.0145	.0144	.0049
Series 5, optimum for roots	0.60	.0208	.0058	.0036	.0195
Knop's solution	0.60	.0063	.0070	.0210	.0083
Series 6, optimum for tops	0.01	.000065	.000378	.000187	.000189
Series 6, optimum for roots	0.01	.000194	.000094	.000062	.000472
Knop's solution	0.01	.00011	.00012	.00035	.00014
Series 7, optimum for tops	2.00	.0169	.0595	.0180	.0734
Series 7, optimum for roots	2.00	.0169	.0895	.0240	.0408
Knop's solution	2.00	.0212	.0238	.0698	.0277

In comparison with the corresponding Knop's solutions of equivalent total concentrations, the best proportions of salts for the three series, judging according to dry weight of tops, differed as follows: The optimum of series 5 contained more phosphate, sulphate, potassium and magnesium, but less calcium and nitrate; that of series 6 contained more magnesium and sulphate, but less of all the other ions; and that of series 7 contained more magnesium, potassium and sulphate, but less calcium, nitrate and phosphate, than does Knop's solution.

As regards root yields, the order of superiority of the different optimal solutions to Knop's solution was different from that just given; series 6 produced the least and series 7 the greatest increase in dry weight of roots, over that given by Knop's solution. Comparing the series with one another, however, series 6 was somewhat superior to series 5, while series 7 was much inferior in this regard.

A comparison of the yields of both tops and roots relative to the controls as unity, in the three series, is presented graphically in figure 15, where the abscissae represent merely the different cultures and the ordinates give the relative yields. The upper graph of each type represents tops, the lower, roots. These graphs show clearly that, excepting to a limited extent in series 7, there is no general correlation between the yields of tops and those of roots.

In series 5 (0.6 per cent. total concentration) there was visible injury to the foliage and a marked reduction in yield of tops in those cultures where the molecular ratio of magnesium to calcium was more than 0.81. No visible injury to the leaves occurred in series 6, but the growth of tops was visibly depressed where the ratios of magnesium to calcium were highest. In series 7 there was not only visible injury and depression of growth where the ratio of magnesium to calcium was more than 0.83, but a much larger proportion of the series was so injured and depressed where the higher proportions of calcium were employed, the injury extending to cultures where the ratio of magnesium to calcium was as great as 8.3. These differences are well shown in the photographs of triangles in each of the different series (figures 5, 8 and 11). The photographs of selected cultures from triangles (figures 6, 9 and 12) show the depression of growth by excess of either magnesium or calcium and the rolled form and bleached condition attending advanced stages of magnesium injury.

Transpiration data for selected cultures, covering a period just preceding harvest, show only a slight correlation of this function with the degree of injury due to excess of magnesium or other cause in series 5, but in series 7 a marked decrease of transpiration relative to yield of the cultures accompanied the cases of severe magnesium injury.

Finally, it is brought out by these series, dealing with the early growth of wheat, that the occurrence of magnesium injury depends upon the proportions of all the salts in the solution rather than upon the mere ratio of calcium to magnesium, as has already been concluded by Gile [13] (page 155) working with rice, and that the yield of dry matter also depends upon the proportions of all the salts. Furthermore, the results here given have shown that the effects, upon the growth of wheat for the first few weeks, of any given set of proportions of the nutrient salts, varies markedly with the total salt concentration of the solution. This conclusion is in harmony with that of Gile [13] and with the results of McCool [13], concerning the antitoxic effect of calcium in overcoming toxicity due to magnesium in different concentrations.

In conclusion, it may be remarked that the general problem opened up by the methods of attack employed in the present paper is a very broad one, and that, even if it be seriously taken up by a number of workers, a long time is apt to elapse before very satisfactory generalizations in this connection may be derived. It is to be remembered that the present study has dealt with only three different total concentrations of the medium, with only one plant form, and with only the first few weeks of its growth. Similar studies of other plant forms and of the later stages of growth of wheat, to bring out the influence upon growth and other physiological processes, exerted by various proportions of the different salts in the medium, must surely be of great value to physiological science.

This study was carried out in the Laboratory of Plant Physiology of the Johns Hopkins University, and the author is deeply indebted, for whatsoever worth the work may possess, to Professor B. E. Livingston, of that Laboratory.

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LITERATURE CITED.

Numbers in brackets throughout the preceding pages refer to the year of publication and to the corresponding numbers which follow authors' names in this list. Where more than one reference to the same year occurs under a given name, these are serially numbered in italics, within the brackets.

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GROWTH OF MAIZE SEEDLINGS IN RELATION TO TEMPERATURE

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ABSTRACT[1]

From the work of former investigators it has appeared that the curve of plant growth in relation to temperature is quite irregular, exhibiting not one principal apex only but, in some instances at least, a double optimum. Whether this apparent double optimum is to be regarded as the result of the interaction of other, uncontrolled, external factors influencing growth, or whether it may be due to internal causes has not been determined.

The published data concerning optimum temperatures for plant growth vary rather widely as to the temperatures indicated as optima. This may be due to the fact that an important factor, that of time, has usually been disregarded in experimentation along this line. Recent researches on optimum temperatures for other physiological activities have shown clearly that a consideration of the time factor is of the utmost importance in such studies. It is thus suggested that the discrepancies of earlier investigations upon temperature optima for growth may be due to the determination of the growth increments at too long intervals. It is also possible that a study involving a wider range of temperatures than has usually been employed, may throw light upon the general problem.

In the experiments here discussed an attempt has been made to keep all the conditions for growth considerably more uniform than has usually been the case with earlier investigations of this sort. Maize seedlings were used, grown practically in darkness and with approximately constant temperature. The relative humidity of the air was always very close to 95 per cent. Due attention was also given to the time factor in its relation to the rates of growth at the different temperatures. Measurements of the growth increments of the shoots were hourly in most cases, and observation was continued for periods ranging in length from 12 to 39 hours. The main results and conclusions brought out by a study of the detailed data presented in the tables, are summarized below.

1. The somewhat widely accepted idea that the curve of growth in relation to temperature shows two optima is not at all substantiated in this work with the shoots of maize seedlings grown in water culture, practically in darkness, and with relative air humidity of 95 per cent.

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SERIAL NO. 5, DECEMBER, 1914.

2. The optimum temperature for growth of shoots of maize seedlings in water culture, *for a 12-hour period,* is shown to be 32°C.

3. The optimum temperature for growth, under these conditions, is found to change as the length of the period of exposure is altered.

4. At high temperatures (31°C. and above), for shoots of maize seedlings under these experimental conditions the initial growth-rate is not maintained, there being a marked falling off in this rate during prolonged periods of exposure.

5. This decrease in the growth rate with prolonged periods at high temperatures makes it necessary to consider the length of the periods for which average growth rates are obtained, in defining the optimum for growth of these shoots. Indeed, it appears that the term *optimum temperature* for growth, in this case at least, is *quite without meaning unless the length of the period of exposure is definitely stated.*

6. The fall in growth rate here brought out is similar to the decrease in rate of certain other physiological processes under the influence of high temperatures during prolonged periods.

7. At temperatures near the minimum (12–14°C.) for the growth of shoots of maize seedlings under the conditions here employed, no decrease in the growth rate is shown, even with rather prolonged periods of exposure.

8. The growth rate at medium temperatures accords with the van't Hoff law, showing a doubling of the rate for each rise of 9° or 10°C.

INTRODUCTION

Rising temperature has long been known to be attended by increased rate of enlargement in growing plants, unless the initial temperature be too high or the temperature change be excessive. It is also generally recognized that the growth curve in relation to temperature, shows three cardinal points: (1) the minimum, a temperature below which no growth is exhibited, (2) the optimum, at which growth is greatest, and (3) the maximum, at which growth again comes to a standstill.

Considerable discussion has recently appeared concerning the true position and the meaning of the optimum temperature for plant growth. Earlier investigators,[2] in their studies regarding the effects of temperature on growth, subjected the plant to a given temperature for a longer or shorter period of time, and measured the increment of growth for that period. The temperature at which the highest growth rate occurred, within the time period chosen, was considered as the optimum temperature for growth. A study of the published data bearing upon the optimum temperature for growth reveals a wide range of variation in its position on the thermometer scale. Sachs [75] placed it at 34°C. for seedlings of flowering plants, and at 33.7°C. for shoots of seedlings of *Zea mais*. Koeppen [70] gives two optima for the shoot of maize, 30° and 33.5°C.; and Sachs [75] states that De Candolle placed it, for the same seedling, at 28°C. Davenport,[3] using Koeppen's data, placed the optimum at 32.4°C.

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[2] Sachs, J., Textbook of botany, translated by A. W. Bennett, assisted by W. T. Thistleton-Dyer. Oxford 1875.

DeVries, H., Matériaux pour la connaissance de l'influence de la température sur les plantes. Arch. Néerland. **5:** 385–401. 1870.

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The most thorough study of the relation of temperature to growth in plants, thus far reported, is that of Koeppen. Working with a number of different seedlings, this author obtained growth curves, in relation to temperature, that are markedly irregular. In many instances these curves show double optima. For maize, Koeppen's curve shows the two optima to lie at 30° and 33.5°C., as above indicated, with a decided drop in the curve, corresponding to intervening temperatures. This curve is here reproduced as figure 1.

The fact that the optimum for growth of the seedling of *Zea mais* as well as for other seedlings, has been placed by different workers at such different temperatures makes further study of this problem desirable. The methods heretofore used in this study have been essentially the same and the accuracy of the observations themselves is not to be questioned; in seeking an explanation for such wide discrepancies as have been noted for maize seedlings we must consequently endeavor to find some condition or conditions not adequately considered by the earlier writers, which may be responsible for the differences in question.

Of course the uncontrolled or neglected conditions which have made for discrepancies in the position of the optimum temperature for growth, as this has been determined, may be supposed to have been related to differences in the physiological nature of the plants employed. For example, different varieties of maize may exhibit different temperature optima because of internal conditions not yet amenable to experimentation; seed of the same variety but produced in different localities or in different years, may furnish seedlings of widely different physiological properties; and, finally, seeds with the same internal properties may give rise to very different seedlings, if germination occurs under sufficiently different environmental conditions. With these suggested possibilities experimentation cannot at present deal and attention is to be first confined to a study of the external, controllable, conditions that may possibly influence the position of the apparent optimum in question.

Clausen's[4] work on the relation of temperature to respiration, that of Chudiakow[5] on fermentation and that of Matthaei[6] on photosynthesis, sug-

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[3] Davenport, C. B., Experimental morphology. New York, 1908.

[4] Clausen, H., Beiträge zur Kenntnis der Athmung der Gewächse und des pflanzlichen Stoffwechsels. Landw. Jahrb. **19**: 893–930. 1890.

[5] Chudiakow, N., Untersuchungen über die alkoholische Gährung. Landw. Jahrb. **23**: 391–532. 1894.

[6] Matthaei, Gabrielle L. C., Experimental researches on vegetable assimilation and respiration. Phil. Trans. Roy. Soc., London (B) **197**: 47–105. 1904.

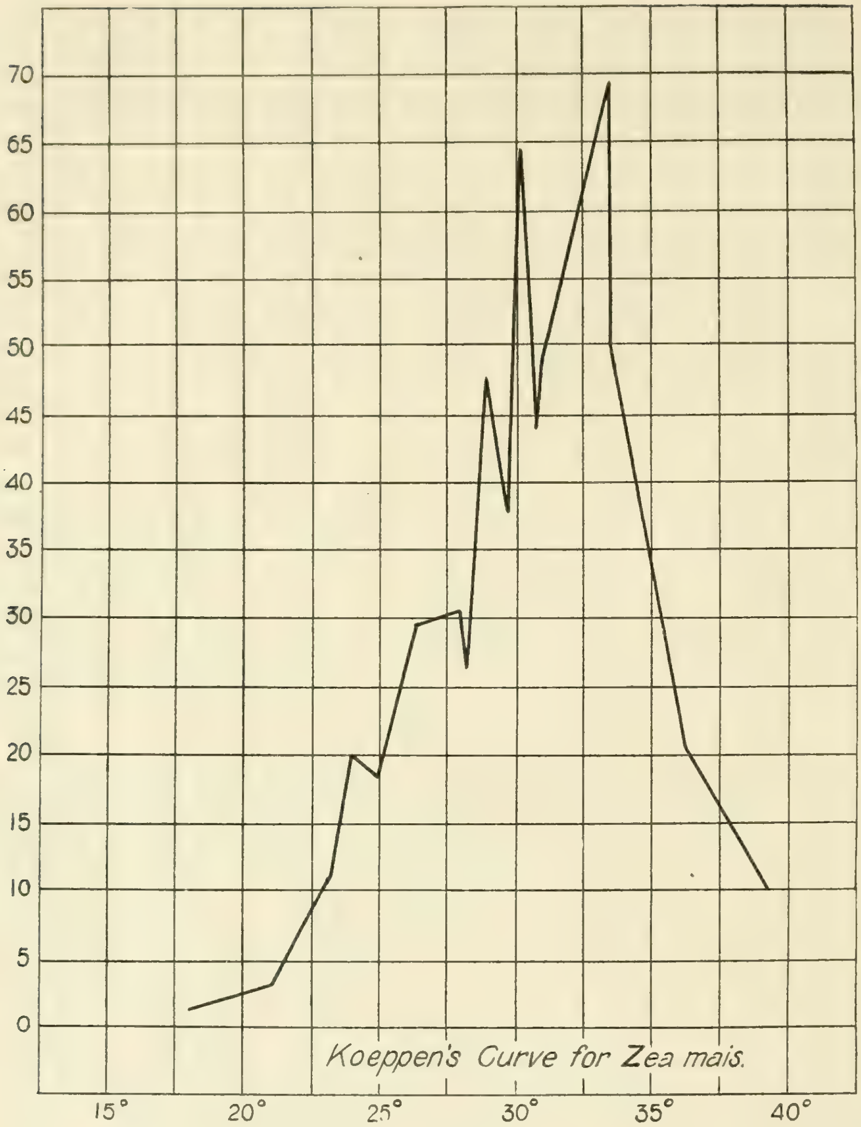

FIG. 1. Graph showing the relation of temperature to growth rate in shoots of maize seedlings, as recorded by Koeppen. The so-called double maximum, with the pronounced depression between the two apices, is shown, together with other irregularities.

gest that the time factor, so important in the consideration of the optimum temperature for the physiological processes just mentioned, may possibly, and even probably, play an equally important rôle in the determination of the apparent optimum temperature for growth. In the important contributions of F. F. Blackman[7] and of Jost,[8] it has been clearly pointed out that this time factor is of fundamental importance in the consideration of the cardinal points of physiological activities in general. In the process of photosynthesis, for example, the initial rate is not long maintained at high temperatures; after a short time, at such temperatures, the rate decreases with more or less rapidity. The same principle holds true for other physiological processes, as respiration, fermentation, etc. Owing to this decrease in the initial rate of physiological action at high temperatures, the position of the optimum shifts with the temperature and with the length of the period of exposure, which is the time elapsing between the application of the given temperature and the first measurement of increment thereafter. Thus, the position of the optimum for such a process as respiration or photosynthesis is found to occur at a higher temperature by an observer who takes his first reading after *one* hour than by another who takes his first reading after *three* hours. In the recorded work on the relation between plant growth and temperature the data were secured from a single measurement in each case, this measurement being obtained usually after the plant had been exposed to the given temperature for a period of 48 hours. Some investigators have measured the increments of growth at much shorter time intervals, but even in such cases a wide range of temperatures has not been employed. Askenasy,[9] in his experiments on the growth of roots of maize, did not go beyond a temperature range of from 28° to 29°C. Godlewski[10] worked only with medium temperatures. True[11] did not carry his experiments beyond a temperature of 30°C., the apparent optimum in his study.

On the whole, it is clear that no very thorough knowledge of temperature optima for plant growth can be attained without much more elaborate study than has yet been carried out, and the considerations just set forth seemed to furnish a new point of view and a promising mode of attack. The present study was undertaken, at the suggestion of Prof. C. F. Hottes, of the University of Illinois, as an investigation of the relations holding between constant temperatures, lengths of exposure periods, and growth rates.

Seedlings of *Zea mais* were chosen for the work, the roots being in nutrient solution and the shoots in circulating air with 95 per cent. of relative humidity, the whole in very weak diffuse light. The experimentation was carried out in the Botanical Laboratory of the University of Illinois. It is with pleasure that I acknowledge my indebtedness to Professor Hottes for suggesting the problem, and for valuable and helpful criticisms, especially in the construction of the apparatus. It is also a pleasure for me to acknowledge many helpful suggestions, as to the presentation of the results, received from Prof. B. E. Livingston, of the Johns Hopkins University.

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[7] Blackman, F. F., Optima and limiting factors. Ann. Bot. **19**: 281–95. 1905.

[8] Jost, Ludwig, Ueber die Reaktionsgeschwindigkeit im Organismus. Biol. Centralbl. **26**: 225–43. 1906.

[9] Askenasy, E., Ueber einige Beziehungen zwischen Wachsthum und Temperatur. Ber. Deutsch. Bot. Ges. **8**: 61–94. 1890.

[10] Godlewski, E., Ueber die Beeinflussung des Wachsthums der Pflanzen durch äussere Factoren. Bull. Internat. Acad. Sci. Cracovie **1890**^{I}: 166-76. 1890. Abstracted in Bot. Centralbl. **47**: 307–9. 1891.

[11] True, R. H., On the influence of sudden changes of turgor and of temperature on growth. Ann. Bot. **9**: 365–402. 1895.

APPARATUS

General. The main apparatus requirements for experimentation such as that here undertaken are the following: the plant chambers must be supplied with air free from laboratory gases; the air humidity must be capable of accurate adjustment and maintenance; it must be possible to maintain constant temperature in the chambers, for relatively long periods of time, and the temperature must be readily and conveniently capable of change when this is desired; and, finally, measurements of the growth increments of the plants must be made with adequate precision and without disturbing the plants or otherwise altering the conditions controlling their growth. The apparatus here employed, designed to secure these features, consisted essentially of (1) the thermostats, (2) the arrangement for constant air humidity, and (3) the plant chambers. These parts will now be briefly described.

The thermostats. A tank of water (approximately 105 cm. long, 75 cm. wide and 30 cm. high) contained submerged Winkler glass coils, through which air was passed to the plant chambers, thus becoming warmed. The same tank contained the saturation chamber, where the air was brought to the proper humidity. Three other thermostat tanks (about 33 cm. long, 20 cm. wide and 33 cm. high, the two square sides of glass) each contained a plant chamber surrounded by water. The glass sides were covered with light-proof curtains (of leatheret, such as is used for carriage covers), excepting while measurements of the plants were being made. These four thermostat tanks were all supplied with electric heating coils, so arranged that the water of the tank was constantly and adequately circulated through them by means of rotating stirrers. An ordinary thermometer suspended in the water showed the temperature, which was controlled in each case by automatic thermo-regulators.[12] The last-named instruments were enclosed in submerged water-tight chambers provided with lead tubes for the electric connections. "Eastman" 4 by 5-inch plate-developing tanks, on the market for use in photography, were found very suitable for these chambers; the lids may be readily removed for adjusting the regulators, and the closure is absolutely water tight.

With this arrangement no difficulty was experienced in maintaining the temperature of the plant chambers constant within a range of fluctuation of

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[12] These were of a type much like that described by Land (Land, W. J. G., An electrical constant temperature apparatus. Bot. Gaz. **52**: 391–9. 1911), with a tongue of steel and hard rubber swinging to and fro with changes of temperature. They were constructed by Mr. G. R. Smith, Electrician of the University of Illinois.

0.5°C. The fluctuation always lay above the temperature given. Thus, in the case of an experiment at 20°, the actual variation of the chamber temperature was from 20° to 20.5°C.

The arrangement for constant air humidity. Air drawn from out-of-doors was first dried by passing through drying chambers with calcium chloride and sulphuric acid. It was next recharged with water vapor to a definite degree by passing through the saturation chamber mentioned above as submerged in the large thermostat tank, this chamber being provided with an 8 per cent. solution of sodium chloride, the proper concentration[13] to give the required air moisture (95 per cent. relative humidity). The main saturation chamber was a glass bottle of about 11 liters capacity, about two-thirds filled with the solution, through which the air was made to bubble.

After being properly charged with moisture in the main saturation chamber the air was conducted through lead tubes to each of the three small thermostat tanks, in each of which it passed through another, smaller, saturation chamber before entering the corresponding plant chamber. These latter saturation chambers served to renew any moisture lost from the air in transit through the tubes, which were unavoidably somewhat cooler than the thermostat tanks; in the small tanks the air was thus rewarmed and recharged with water vapor, to the requisite temperature and humidity. From the plant chambers the air was withdrawn, by means of one or two water aspirators (arranged to be used singly or combined), and was discharged outside of the apparatus. The aspiration tube was joined to each of the three plant chambers, and the rate of air movement through these was regulated by means of mercury manometers connected with the chambers, and a series of stop-cocks in the supply tubes.

The plant chambers. The plant chambers were constructed of glass jars 24 cm. high by 12 cm. square, with a short cylindrical neck above, the diameter of the neck being but little less than that of the prismatic basal portion. The glass neck was prolonged upward (15 cm.) by a cylinder of tinned sheet iron, attached by plaster of Paris, the free surface of the latter being covered with a hard cement of rubber-resin mixture. The metal portion of the chamber was covered inside and out with black acid-proof, non-fuming paint ("Probus" brand, made by Wolff and Dolan, San Francisco, Calif.) Two nipples near the upper edge provided passage for the two air tubes. Of these latter, the exit tube reached nearly to the bottom of the chamber, the other (air inlet) merely through the wall. A friction lid closed the main opening at the upper end of the metal cylinder, the joint being further sealed and rendered air-tight with a mixture of modelling clay and petrolatum (vaseline), such as is recommended by Crocker.[14] This lid could be readily removed and replaced, thus furnishing ready access to the chamber, for inserting and removing the culture bottles.

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[13] If H denotes percentage of relative humidity, then $H = 1 - na$, wherein n is the weight of solute in 100 g. of water, and a is a constant varying with the nature of the salt employed. This constant, a, is 0.00601 for sodium chloride. (See Davenport [08], page 351. Also see:—Lesage, P. Recherches expérimentales sur la germination des spores du Penicillium glaucum. Ann. Sci. Nat. Bot. VIII. **1**: 309–22. 1895. Lesage derives the value of a from work of Wüllner:—Wüllner, A, Versuche über die Spannkraft des Wasserdampfes aus wässerigen Salzlösungen. Ann. Phys. und Chem. **103**: 529–62. 1858. **105**: 85–117. 1858). According to this formula, a solution of sodium chloride containing 8 g. of salt to 100 g. of water should give 95 per cent. of relative humidity in air with which the solution is in equilibrium: $H = 1 - 8 \times 0.00601 = 0.95$.

The plant chambers were immersed in the water of the thermostats, the metal neck remaining above the water level. When equipped for an experiment each chamber contained a thermometer and Lambrecht polymeter, besides the culture bottle and its two plants. Readings of the temperature and relative humidity of the chambers were made hourly and recorded with the hourly increments of growth. The Lambrecht polymeter (hair hygrometer) in each plant chamber indicated that the relative air humidity fluctuated not more than 1.5 per cent. of relative humidity in either direction, while the apparatus was in operation. The actual humidity as indicated by the Lambrecht instruments, varied from a minimum of 93 to a maximum of 96 per cent., making it safe to consider it as approximately 95 per cent.

Such slight changes in temperature and humidity conditions as were observed exhibited no relation to the growth fluctuations, showing that the constancy of temperature and humidity control was adequate for the work in hand.

METHODS

Manipulation of apparatus. In beginning an experiment the thermostats and the plant chambers were first brought to the temperature desired. Sufficient time was allowed for the glass walls of the plant chambers to attain the temperature of the water of the thermostats. When low temperatures were employed the water in the thermostats was first cooled by adding ice, the thermo-regulators maintaining the somewhat higher temperature desired. On cold days it was found advantageous to open the windows and thus to cool the room below the temperature required for the thermostat.

After the desired temperature had been attained within the plant chambers, the plants, polymeter and thermometer were put in place and the top was securely closed. Then the aspirators were started and adjustment made until a constant and uniform flow of air through each chamber was attained. An interval of 2 hours was allowed to elapse for the culture bottle and its solution, the plants, the polymeter and the thermometer to attain the temperature of the chamber, and for the plants to accommodate themselves to the new conditions. After this interval measurements of the growth increments of the shoots were started and continued, at intervals, throughout the experiment.

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[14] Knight, L. I., and Crocker, W., Toxicity of smoke. Bot. Gaz. **55**: 337–71. 1913. Page 339.

The plants were practically in darkness during the period of experimentation; only very weak diffuse day-light, which might penetrate far into the room, and then through the water of the thermostat and the glass walls of the plant chamber, reached them by day. Light for the measurements was provided by continuously operated electric bulbs, one immediately in front of, and another behind, the thermostat, the opaque curtains being raised during each observation. The plants were never directly exposed to this light for a period longer than 40 seconds. Under these conditions the seedlings were always erect, and absolutely no day and night periodicity in growth was at any time apparent. Slight greening occurred.

The measurements were made with telescopes with cross hairs, like cathetometers. A scale graduated in half-millimeters, placed immediately behind the plant, permitted ready and sufficiently accurate measurements of the height of the tip of the shoot above the zero of this scale.

Preparation of seedlings. "Reed's Yellow Dent" maize, of the previous year's harvest, grown on the Illinois Agricultural Plots, was used. The seed was uniform and of good quality, showing a percentage of germination such as agriculturists regard as good. The grains were soaked in water for about 10 hours, and were then allowed to germinate in sphagnum moss, in a basement dark room (free from gas pipes) with a temperature of from 27° to 29°C.

When the seedlings had attained a sufficient size for experimentation (the shoots being from 10 to 12 mm. high) they were transferred on the evening preceding the day of the experiment, to wide-mouthed bottles (125 cc. capacity) of nutrient solution. These bottles were provided with 2-perforate, tightly fitting, paraffined, flat cork stoppers. The roots and seed of a seedling were passed through each perforation and each plantlet was held in place by means of the usual cotton packing above the seed. The bottles were wrapped in black paper, opaque to light. The culture bottles, each with its two seedlings, remained in the dark room during the night and the growth rates of the plants were approximately determined for this period, in order to eliminate any individuals which appeared to be abnormal. They were placed in the plant chambers on the morning of the following day. The nutrient solution employed was that known as Tollen's solution.[15] This solution is prepared in two parts, A and B. Part A consists of 2.05 g. $MgSO_4$ $(7H_2O)$ dissolved in 35 cc. of water. Part B contains 4 g. $Ca(NO_3)_2$ $(4H_2O)$, 1 g. KNO_3 and 1 g. KH_2PO_4, all dissolved in 35 cc. of water. The complete solution is made by combining 10 cc. of part A and 35 cc. of part B with 980 cc. of water and adding to this a few drops of an aqueous solution of ferric chloride.

The water used in the nutrient solution was first distilled, with a still having a block tin condenser. It was redistilled in hard glass. Finally it was shaken with thoroughly washed carbon ("G. Elf" brand carbon black), as recommended by Livingston[16] and by Schreiner and Skinner,[17] to remove traces of toxic material which might be present. A fresh solution was prepared for every experiment.

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[15] Pfeffer, W., The physiology of plants, translated by A. J. Ewart. 1: 420. Oxford, 1900.

POSSIBLE DISTURBING CONDITIONS

In comparing growth rates under different temperature conditions it is of course essential that all plants be similarly treated, excepting for the single condition, temperature, the influence of which is to be studied. Since any change in the environment may be expected to have some effect upon subsequent growth, at least for a limited period after the change, it became necessary to determine, by preliminary experiments, how long the disturbance of growth might be effective, which is caused by the change of bringing the seedlings under the experimental conditions. Two environmental changes which might not be without influence upon later growth, and which were unavoidable in the starting of the experiments, require attention here, the change of medium and the change of temperature. These will next be considered.

Change of medium. The transfer of the seedlings from sphagnum moss to the bottle of nutrient solution necessitated a handling and also a change of medium, which produced a decided disturbance in growth, a result which is in accord with similar observations of Askenasy [90] and of many other experimenters. An interval of 12 hours after the transfer was found amply sufficient, however, to allow the seedling to accommodate itself to the new conditions, and no effect of the transfer itself was apparent after that interval. The transfer was made as rapidly and carefully as possible, so that the shock incidental to handling was reduced to a minimum.

Change of temperature. In the experiments to be considered below, the temperature at which the growth of the seedlings was studied was often several degrees higher or lower than the temperature at which they had grown. It was thus necessary to determine the length of period required for the recovery of the plant from the temporary depression or acceleration of growth, incident to the initial change in temperature.

The effects of sudden changes of temperature on plant growth have been studied by a number of investigators. One of the first of these was Koeppen [70], who observed that an abrupt change of temperature, in either direction, brought about a lowering of the growth rate. He maintained that it is the fact of a sudden change, rather than the direction or amount of change, that affects growth.

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[16] Livingston, B. E., Further studies on the properties of unproductive soils. U. S. Dept. Agric. Bur. Soils Bull. 36. 1907.

[17] Schreiner, O., and Skinner, J. J., Ratio of phosphate, nitrate and potassium on absorption and growth [*sic*]. Bot. Gaz. **50**: 9–30. 1910.

Pedersen[18] repeated the experiments of Koeppen and obtained results which led him to a diametrically opposite conclusion. He exposed seedlings of *Vicia faba* to sudden and to gradual changes of temperature between 10° and 20°R. (12.5° and 25°C.) and reported that no evidence of any retardation of growth was manifested. He found the growth rate to depend solely on the temperature itself, the simple fact of change being without influence.

This matter was again taken up by Askenasy [90], who confined his observations to roots of maize seedlings. He found that the influence due to the fact of sudden temperature change depends upon whether the first temperature, from which the transfer is made, lies above or below the minimum temperature for growth. When the first temperature still permitted growth, change to a higher temperature was followed by an immediate resumption of the rate of growth characteristic of the higher temperature. When, however, the first temperature was near the point where growth was inhibited, change to a higher temperature was followed by a more or less tardy resumption of the normal growth rate.

The results of True [95], obtained in Pfeffer's laboratory, mainly confirm those of Askenasy. He found that the effect upon growth, of a sudden change of temperature, depends upon "the position of the lower limit." This writer concludes as follows:

> Following a sudden fall or a sudden rise of the temperature between 18°–21°C. and 0.5°–1.5°C. as extremes, the first effect seen is a slight turgor-change due to physical causes, producing, or tending to produce, a shortening in length if the temperature be lowered; or in case the temperature be raised, producing an elongation. Following this mechanical action a period of depressed growth usually follows. The duration of the depression-period depends on the position of the lower temperature limit and on the length of time of exposure to this temperature.

With higher temperatures True found that every change from 30° to 18°C. is followed by a reduction of the growth-rate during the ensuing one-fourth hour. Further effects plainly due to the change are not to be traced in the later growth. It increases or diminishes according to the internal conditions prevailing. The transfer from 18° to 30°C. is always followed by an increased amount of elongation during the ensuing fifteen minutes. As before, the growth-rate gives no further evidence of being influenced by the temperature-change. In both cases, the traceable effects of the change disappear within fifteen minutes.

The experiments of Price[19] on the influence of temperature changes as such on the rapidity of development of flower buds of fruit trees may be mentioned here. Price found that "the retardation of development by cold is altogether temporary and directly proportional to the time during which the low temperature prevails." That is, there was no observable effect of temperature change as such, in his experiments.

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[18] Pedersen, R., Haben Temperaturschwankungen einen ungünstigen Einfluss auf das Wachsthum? Arb. Bot. Inst. Würzburg **1**: 563–83. 1874.

[19] Price, H. L., The application of meteorological data in the study of physiological constants. Ann. Report Virginia Agric. Exp. Sta. **1909–10**: 206–12. 1911.

Some tests of the influence of sudden change of temperature, amounting to five degrees, on the rate of growth of maize seedlings, were made in the present study. The temperature was first kept at 25°C. for 6 hours and was then raised, within a period of 5 minutes, to 30°C. Results of such tests are presented in table I, which is self-explanatory.

The data of table I show that the rate of growth was markedly increased during the second and third hours following the change of temperature from 25° to 30°C., the rate then falling to the characteristic magnitude for the higher temperature. This result differs somewhat from those obtained by Askenasy [90] and by True [95]. As has been noted, the earlier of these two authors found no influence exerted by the fact of such a temperature change as this, *per se*. True found that such a change, from lower to higher temperature, resulted in an initial rise in the growth rate to a magnitude higher than the normal one for the higher temperature, which was very soon followed, after but 15 minutes, by a rapid fall to that normal. The essential difference between the results of True and those of the present study lies in the longer period required by my plants to assume the growth rate characteristic of the new temperature. This difference may possibly be accounted for by a difference between methods employed for changing the temperature.

TABLE I

Hourly growth increments (hundredths of a millimeter) for shoots of maize seedlings growing in practical darkness, with 95 per cent. relative air humidity, for 11 hours; the temperature being maintained at 25°C. during the first 6 hours, after which it was changed[a] *and maintained at 30°C. during the remaining 5 hours.*

NO. OF HOUR	NUMBER OF PLANT									AVERAGE	TEMPERATURE, C.
	1	2	3	4	5	6	7	8	9		
1	50	25	75	25	50	75	75	50	50	53	25°
2	25	50	75	50	50	50	75	75	50	55	
3	75	75	75	75	50	50	50	75	50	64	
4	75	75	75	75	75	75	50	50	75	69	
5	50	50	100	75	75	50	75	75	100	72	
6	50	75	100	75	75	75	75	75	100	78	
7	75	125	125	75	100	75	75	100	100	94	30°
8	100	125	175	150	150	150	150	125	150	142	
9	100	100	150	150	125	100	125	125	125	122	
10	100	100	125	125	100	75	100	125	100	105	
11	100	100	175	150	100	75	100	100	75	108	

[a] The temperature change occurred within a period of 5 minutes.

With the apparatus here used the temperature of the plant chamber could be suddenly raised (in less than 5 minutes) the five degrees required, but the bottle and nutrient solution assumed the new temperature but slowly. The nutrient solution attained the higher temperature in considerable less than 2 hours, however. Whatever may have been the reason for the difference between True's results and those here recorded, it is clear from table I that the effect of temperature change itself, from lower to higher, had ceased to be appreciable, in the present study, by the end of the initial 2-hour period allowed for the plants and their surroundings to come into physical and physiological equilibrium.

Since it was intended to study the growth rate at temperatures lower as well as higher than that at which the seedlings had grown, a further series of experiments was undertaken to determine the special influence of changes from higher to lower temperature. Two lots of seedlings were grown at 20° and 29°C., respectively. A number from each lot were then placed in the plant chambers at 25°C., and the increments of growth measured. The results are shown in table II.

TABLE II

Hourly growth increments (hundredths of a millimeter) for shoots of maize seedlings growing in practical darkness, with 95 per cent. relative air humidity, for 7 hours, at a constant temperature of 25°C.; the two groups of seedlings having been previously grown with constant temperatures of 29° and 20° respectively.

NO. OF HOUR	GROUP I Seedlings grown at 29°C.					GROUP II Seedlings grown at 20°C.				
	1	2	3	4	AVERAGE	1	2	3	4	AVERAGE
1	25	25	50	25	31	75	100	100	125	100
2	50	75	75	75	69	50	75	50	100	69
3	50	50	75	75	62	75	75	50	75	69
4	50	75	75	75	69	75	75	75	75	75
5	75	50	75	100	75	75	50	75	75	69
6	75	75	100	100	87	75	75	75	75	75
7	75	100	100	75	87	100	75	75	50	75

An inspection of table II shows that the change from lower to higher temperature (20° to 25°C.) resulted in a special acceleration of growth similar to that brought out in table I, but this effect vanished in the present case after only a single hour. For the change in the other direction (from 29° to 25°C.) the data of table II show that the growth rate for the first hour following the change was lower than the final, nearly uniform rate for 26°. It thus appears that the special or "shock" influence of change from higher to lower temperature may be considered as not effective after the first hour subsequent to the change.

From the experimental data recorded in tables I and II it appears that the 2-hour period allowed to elapse in this study, between the initial temperature change and the beginning of growth measurements, was amply sufficient to avoid growth rates that might have been due to the temperature change *per se*, in whichever direction the change occurred. As a further precaution in this connection, besides the 2-hour period of adjustment after the cultures were placed in the plant chamber, the culture bottle and its nutrient solution were gradually brought to the new temperature before the transfer, whenever the experiment called for a marked difference between the cultural and the experimental temperatures.

NORMAL GROWTH

In experimental work involving a large number of different individual plants the amount of variation due to unknown conditions is often very great. In the present studies there appeared considerable individual variation in the rates of growth of different plants under apparently the same environmental conditions. Occasionally a seedling exhibited a rate of growth far above or far below the general average. As is usual in such experimentation as this, such widely erratic individuals were rejected. Askenasy [90] favors such a procedure. In discussing the question here raised, he says (page 63):

> Es ist sehr wichtig, bei vergleichenden Versuchen nur mit gesunden Wurzeln zu arbeiten. Das einzige sichere Zeichen der Gesundheit einer Wurzel ist ein entsprechendes Wachsthum. Ich habe darum immer das Wachsthum der Wurzeln erst eine Zeit lang bei einer dem Optimum nahen Temperatur (26–29°) beobachtet und alle Wurzeln, die unter 1.7 mm. stündlichen Zuwache zeigten, verworfen. Pedersen bemerkt allerdings, dass "es nicht erlaubt wäre, jede langsam wachsende Wurzel als krank und abnorm zu betrachten, bloss deshalb weil sie langsam wächst." Ich bin aber anderer Ansicht. Viele Umstände, die auf das Wachsthum der Wurzeln ungünstig einwirken, sind uns nicht genügend bekannt, wir nehmen sie nur an ihren Folgen wahr, und wir werden sicherer gehen, wenn wir abnorm langsam wachsende Wurzeln verwerfen.

True [95] evidently eliminated those plants of his cultures that showed an abnormally rapid or slow growth. He states (page 368): "In the investigation only those specimens were used which, by at least an average growth, indicated a normal condition."

The seedlings used in the present studies were grown from seeds taken from selected ears of the same progeny as far as possible. Also, as far as possible, the plants of each series were from grains taken from the same ear, those from tip and butt being rejected. They were all grown under apparently identical external conditions and only those of the same age and approximately the same height were chosen for the tests. (See Pfeffer[20] in this connection.) All seedlings with shoots more than 12 and less than 10 mm. in length were rejected. Finally, before the seedlings were placed in the plant chambers, they were grown in nutrient solution for from 8 to 10 hours at a temperature of from 27° to 29°C., with measurement of the growth rate, and all whose rate deviated widely from the average of a large number were thrown out.

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[20] Pfeffer, W., The physiology of plants, translated by A. J. Ewart. **2: 15, 79.** Oxford, 1903.

RESULTS

General plan of presentation. In the presentation of the numerical data, which follows, there are always two time periods involved, the period of exposure to the given temperature and the period of observation or time increment. The period of exposure is the length of time from the beginning of the growth measurements to the end of the experiment or to any chosen time before this. The periods of observation are the periods elapsing between the consecutive growth readings; thus the period of exposure is the sum of the lengths of all the periods of observation considered.

It will be observed that an experiment having a total exposure period of 32 hours, for example, with determination of hourly growth increments, furnishes data for exposure periods of 1, 2, 3, etc., up to 32 hours, and the average hourly growth rate may be readily determined for any or all of these 32 different lengths of exposure period. The growth increment for any period of observation (time increment) is the difference between the measurement taken at the beginning and that taken at the end of that period of observation. The first measurement was always made at 10.00 a.m., 2 hours after the plants were placed in the plant chambers. For convenience in presentation, all growth increments are here stated in terms of hundredths of a millimeter.

As has been mentioned, the readings were made hourly in most cases, so that the time increments are usually single hours and the growth increments correspond to the same periods. With temperatures near the minimum and with those near the maximum for growth, however, hourly growth increments are so small as to render the probable error of measurement relatively great. In these cases, and in a few others, the period of observation was 3 hours. In a very few cases it was still longer.

Tests were made for nearly all temperatures from 12° to 43°, by differences of 1°C. For temperatures from 20° to 30° the total period of exposure was 12 hours; for other temperatures the total period was longer in many cases, ranging in length to 21 or even to 39 hours.

In the tables which follow, where the data for the individual plants are given, the cultures are numbered serially and the two plants of each culture are distinguished by the letters a and b. Thus plants 1a and 1b, 2a and 2b, etc., are pairs which were in the same culture, and hence in the same plant chamber at the same time. These detailed observations are given in order that the kind of fluctuations met with in the increment values may be placed on record.

Increments of growth for temperatures from 12° to 43°C. The great mass of individual growth increments need not be set forth here, but three representative groups of these data, to show the extent of their variation due to unknown causes and to illustrate their general character, are given in tables III, IV and V. Table III refers to low temperatures (12° to 15°), where growth is very slow; table IV refers to medium temperatures (30° to 34°), where growth is most rapid; and table V refers to high temperatures (40° to 43°), where growth is again very slow. The time increments, or periods of observation, have, generally, a length of a single hour for the medium temperatures, and one of 3 hours for the low and for the high temperatures, this difference being for reasons already given. The last column in each table gives the averages of the growth increments (for the corresponding time increments) for all the plants used.

It is at once to be noted, from tables III, IV and V, that the individual, unexplained variations which are apparent here are most pronounced with the low and with the high temperatures; as the limits for growth are approached the individual differences between the seedlings (their internal conditions) become more influential in conditioning the growth rate.

The average growth increments for the different observation periods, for temperatures from 12° to 43°, derived as in tables III, IV and V, are all brought together in table VI. The mean hourly rates are given in parenthesis, for all observation periods of more than 1 hour, and for periods of 1 hour the average increments themselves are mean hourly rates.

Aside from irregular variations in the hourly rates, it is obvious from table VI that, with increasing temperature, these rates increase from a first minimum (3, for the second 3-hour period at 12°) to a maximum (167, for the twenty-seventh hour at 31°) and then decrease to a minimum of zero (for the seventh 3-hour period at 41° and at 42°, and for the sixth 3-hour period at 43°). The magnitude of any mean hourly rate is seen to depend upon two conditions, (1) the temperature employed, and (2) the time period during which the plants have been previously subject to that temperature.

The last statement is the main general result of the present study and it does not appear to have been seriously considered heretofore, in the literature of the relation of plant growth to temperature. This generalization would have been more thoroughly established had the observations been continued longer with the temperatures below 31°, for there is no clear evidence, in the data at hand, that longer continued observation might not have discovered higher hourly rates than those recorded. For the temperatures above 31° the data are adequate to establish the point in question, however. The highest hourly rate (150) for 32° was obtained in the eleventh hour of the experiment; the highest (142) for 33° occurred in the fifteenth and nineteenth hours; the highest (144) for 34° occurred in the nineteenth hour; that (120) for 35° occurred in the seventh hour; that (104) for 36° occurred in the ninth

TABLE III

Increments of growth (hundredths of a millimeter), for shoots of maize seedlings, generally for time increments of 3 hours, at temperatures of 12° to 15°C., the latter maintained constant during the entire period of exposure, in each case. The individual plants, for each temperature, are numbered 1a, 1b, 2a, etc.

TEMPERATURE, C.	SERIAL NUMBER OF OBSERVATION	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	AVERAGE
12°	1 (3)[a]	50	25	25	75	50	50	...	...	...	...	...	...	46
	2 (3)	00	25	25	00	00	00	...	...	...	...	...	...	8
	3 (3)	25	25	00	25	50	50	...	...	...	...	...	...	29
	4 (3)	00	25	25	00	00	100	...	...	...	...	...	...	25
13°	1 (3)	25	50	25	50	50	50	...	...	...	...	...	...	42
	2 (3)	25	00	25	25	25	00	...	...	...	...	...	...	17
	3 (3)	25	25	25	25	25	25	...	...	...	...	...	...	25
	4 (3)	25	25	25	25	75	25	...	...	...	...	...	...	33
	5 (3)	50	50	25	50	50	50	...	...	...	...	...	...	46
	6 (8)	100	150	100	150	200	75	...	...	...	...	...	...	129
	7 (2)	25	25	75	...	...	25	...	...	...	...	...	...	38
	8 (3)	75	25	00	25[b]	50[b]	25	...	...	...	...	...	...	31
	9 (3)	75	50	25	75	75	25	...	...	...	...	...	...	54
	10 (4)	50	.	75	50	100	50	...	...	...	...	...	...	65
14°	1 (3)	50	50	50	50	50	100	...	...	...	...	...	...	58
	2 (3)	25	25	25	25	25	50	...	...	...	...	...	...	29
	3 (3)	50	75	50	...	50	50	...	...	...	...	...	...	55
	4 (3)	50	50	50	75[c]	50	75	...	...	...	...	...	...	55
	5 (3)	75	75	50	50	50	75	...	...	...	...	...	...	63
	6 (8)	200	200	200	75	150	225	...	...	...	...	...	...	175
	7 (3)	50	75	100	75	75	75	...	...	...	...	...	...	75
	8 (3)	100	100	100	50	75	50	...	...	...	...	...	...	79
	9 (3)	25	75	...	...	100	100	...	...	...	...	...	...	75
	10 (3)	75	100	200[d]	75[d]	100	100	...	...	...	...	...	...	94
15°	1 (3)	50	50	50	75	150	125	25	50	50	50	25	50	63
	2 (3)	25	25	75	25	25	25	25	25	25	25	75	75	38
	3 (3)	50	100	75	100	75	75	75	50	50	100	25	50	69
	4 (3)	75	75	100	75	50	75	50	75	75	100	50	75	73
	5 (3)	75	75	75	100	75	75	100	100	100	100	75	100	88
	6 (1)	25	25	00	25	25	25	...	...	...	...	...	...	21
	7 (2)	...	...	...	...	...	...	100[e]	75[e]	100[e]	100[e]	100[e]	50[e]	..
	8 (7)	...	...	...	...	...	...	200	200	175	350	250	275	242
	9 (3)	...	...	...	...	...	...	100	100	100	150	75	100	104
	10 (3)	..	..	...	...	...	..	75	100	100	175	150	.....	120
	11 (3)	...	...	...	...	...	...	100	100	125	125	75	175[f]	105

[a] The length of the observation period (in hours) follows its number, in parenthesis.

[b] For observation periods 7 and 8 combined, 5 hours, not included in average.

[c] For observation periods 3 and 4 combined, 6 hours, not included in average.

[d] For observation periods 10 and 11 combined, 6 hours, not included in average

[e] For observation periods 6 and 7 combined, 9 hours.

[f] For observation periods 10 and 11 combined, 6 hours, not included in the average.

TABLE IV

Increments of growth (hundredths of a millimeter) for shoots of maize seedlings, generally for time increments of one hour, at temperatures of 31° to 34°C., the temperature maintained constant, in each case, for the entire period of exposure. The individual plants, for each temperature, are numbered 1a, 1b, 2a, etc.

TEMPERATURE, C.	SERIAL NO. OF OBSERVATION PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	AVERAGE
31°	1	25	50	125	50	50	25							54
	2	25	50	50	100	75	50							58
	3	75	100	100	75	125	100							96
	4	125	100	100	100	100	100							104
	5	150	100	100	175	150	125							133
	6	100	100	100	150	100	125							123
	7	150	100	100	150	125	125							125
	8	150	75	125	125	75	125							113
	9	100	125	125	150	125	125							125
	10	150	100	100	150	125	150							129
	11	150	100	100	150	100	100							117
	12	150	100	150	225	150	150							154
	13	125	100	75	200	100	175							129
	14	125	100	125	200	150	125							138
	15	150	150	125	150	100	150							138
	16	150	100	75	150	100	150							121
	17	150	125	125	150	75	150							129
	18	100	125	150	225	175	125							150
	19	150	150	125	150	150	125							142
	20	125	150	150	250	125	150							158
	21	125	100	150	200	100	150							138
	22	150	125	125	175	125	150							142
	23	125	150	150	175	125	125							142
	24	125	175	125	150	125	125							138
	25	150	150	100	150	175	125							142
	26	175	150	200	150	125	150							158
	27	175	150	175	225	150	125							167
	28	150	150	200	175	150	150							163
	29	150	125	200	175	125	175							158
	30	150	125	225	150	125	175							158
	31	150	125	150	150	125	175							146
	32	150	150	150	150	100	150							142
	33	150	125	175	175	125	125							146
	34	150	125	175	150	125	150							146
	35	150	175	150	175	125	150							154
	36	150	150	125	200	150	150							154
	37[a]	400	450	375	425	400	425[a]							413
32°	1	75	75	75	75	50	75							71
	2	75	75	75	75	75	75							75
	3	75	75	100	75	75	100							83

TABLE IV—Continued

TEMPERATURE, C.	SERIAL NO. OF OBSERVATION PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	AVERAGE
32°	4	100	75	100	100	100	125	.....	.....	.....	.....	.....	.....	100
	5	125	100	100	100	75	125	.....	.....	.....	.....	.....	.....	104
	6	150	125	100	125	100	125	.....	.....	.....	.....	.....	.....	121
	7	100	100	100	125	100	150	.....	.....	.....	.....	.....	.....	113
	8	125	125	125	125	100	150	.....	.....	.....	.....	.....	.....	125
	9	125	125	125	100	100	150	.....	.....	.....	.....	.....	.....	121
	10	125	125	125	100	125	150	.....	.....	.....	.....	.....	.....	125
	11	125	150	150	125	175	175	.....	.....	.....	.....	.....	.....	150
	12	125	150	150	125	175	125	.....	.....	.....	.....	.....	.....	142
	13	125	150	125	150	125	150	.....	.....	.....	.....	.....	.....	138
	14	150	150	150	150	125	125	.....	.....	.....	.....	.....	.....	142
	15	125	150	150	100	150	125	.....	.....	.....	.....	.....	.....	133
	16	125	150	150	150	175	125	.....	.....	.....	.....	.....	.....	146
	17	125	125	150	150	175	150	.....	.....	.....	.....	.....	.....	146
	18	125	125	150	175	150	125	.....	.....	.....	.....	.....	.....	142
	19	125	125	125	150	150	125	.....	.....	.....	.....	.....	.....	133
	20	150	125	125	150	150	125	.....	.....	.....	.....	.....	.....	138
	21	125	150	125	175	125	150	.....	.....	.....	.....	.....	.....	142
	22[a]	450	400	450	450	425	425	.....	.....	.....	.....	.....	.....	433[a]
	23	450	450	425	425	400	425	.....	.....	.....	.....	.....	.....	429
	24	400	400	375	400	425	425	.....	.....	.....	.....	.....	.....	404
	25	400	400	425	400	450	425	.....	.....	.....	.....	.....	.....	417
	26	450	500	400	450	350	375	.....	.....	.....	.....	.....	.....	421
	27	400	400	250	325	275	375	.....	.....	.....	.....	.....	.....	338
33°	1	50	50	50	75	75	100	75	25	50	50	50	50	58
	2	75	75	100	75	75	50	75	50	50	50	50	25	63
	3	100	125	75	100	75	25	100	75	50	100	100	75	83
	4	100	75	75	75	125	75	100	75	75	100	100	75	88
	5	75	100	75	100	75	100	100	100	75	100	125	100	94
	6	100	100	100	125	125	150	100	100	100	125	125	100	113
	7	100	100	125	200	100	100	150	100	100	100	125	100	117
	8	125	100	75	100	100	125	125	125	100	125	125	100	110
	9	125	100	125	125	125	125	125	125	125	150	125	150	127
	10	100	125	100	150	125	100	125	100	100	125	150	125	119
	11	100	125	100	125	100	100	100	100	125	125	125	100	110
	12	125	125	100	150	150	100	125	125	150	150	125	125	129
	13	100	125	150	175	150	100	100	125	150	125	150	125	131
	14	100	100	125	175	175	100	125	100	150	125	125	150	129
	15	125	125	125	250	175	125	125	100	125	150	125	150	142
	16	125	125	100	150	175	100	125	125	125	125	125	150	129
	17	100	125	125	175	175	200	125	100	150	125	125	150	140
	18	100	125	75	75	150	50	150	100	125	150	125	100	110
	19	125	150	125	225	200	125	125	125	125	125	100	150	142
	20	100	100	125	125	150	125	200	125	125	125	125	150	131
	21	100	100	100	150	150	125	100	100	125	125	150	100	119

TABLE IV—Continued

TEMPERATURE, C.	SERIAL NO. OF OBSERVATION PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	AVERAGE
34°	1	100	25	75	75	25	75	25	75	75	25	50	75	58
	2	100	25	50	25	50	50	50	125	75	50	50	25	48
	3	25	75	50	25	150	75	50	100	75	100	50	75	71
	4	50	100	100	50	100	75	50	100	100	100	75	100	83
	5	100	100	100	25	150	125	100	100	125	100	100	75	100
	6	125	125	100	50	100	100	75	100	125	125	75	100	100
	7	125	100	125	75	125	150	125	175	150	150	75	125	125
	8	100	100	100	75	125	100	75	175	150	125	100	125	113
	9	100	125	75	75	150	100	100	150	150	150	75	100	113
	10	150	175	150	100	125	125	75	175	125	100	100	100	125
	11	100	100	100	100	125	125	150	150	150	150	75	125	121
	12	100	150	100	75	100	125	25	175	125	125	100	100	108
	13	75	125	125	100	175	125	150	125	125	100	75	75	115
	14	100	125	75	75	125	125	25	150	150	125	75	100	104
	15	100	150	75	100	100	125	125	150	100	150	75	100	113
	16	150	150	100	125	200	150	75	175	125	175	75	100	133
	17	125	175	100	125	100	125	125	150	125	150	125	100	127
	18	125	125	50	100	150	100	100	125	150	125	75	75	108
	19	150	150	125	150	125	175	150	150	150	150	150	100	144
	20	175	150	150	125	175	125	100	125	100	175	75	75	129
	21	100	150	25	100	100	100	100	75	125	125	50	75	94

[a] Period 37 for 31° and periods 22 to 27 for 32° were 3 hours in length; note that the growth increments given, and the averages, are 3-hour rates.

hour; that (88) for 37° occurred in the seventeenth and nineteenth hours; that (84) for 38° occurred in the ninth hour; that (60) for 39° occurred in the third hour, etc. Obviously there is a tendency evident, for the highest hourly mean to be exhibited at an earlier hour with higher and higher temperature. At the same time, this highest mean is progressively lower with higher temperature.

From these results it is clear that, to define the temperature with which a given growth rate is to be expected, it is absolutely necessary to state the length of time during which the plants have been subjected to the action of the temperature in question.

Average hourly growth rates for various periods of exposure. Turning now to the question of determining the optimum temperature for prolonged growth, it follows directly from what has just been brought out that such an optimum cannot be defined without reference to the period of exposure, but, to make this conclusion more obvious and to bring the presentation of my data into conformity with other published work on this subject, the increments of growth, for each temperature, have been treated as though the

TABLE V

Increments of growth (hundredths of a millimeter) for shoots of maize seedlings, for time increments of three hours, at temperatures of 40° to 43°C.; the temperature maintained constant, in each case, for the entire period of exposure. The individual plants, for each temperature, are numbered 1a, 1b, 2a, etc.

TEMPERATURE, C.	SERIAL NO. OF OBSERVATION PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	AVERAGE
40°	1	100	75	75	75	75	100	75	75	81
	2	125	150	125	175	125	125	100	100	128
	3	100	100	125	100	100	100	100	100	103
	4	50	100	50	25	50	100	75	75	66
	5	100	100	75	100	75	50	75	50	78
	6	75	75	75	50	50	50	50	75	63
	7	100	75	50	50	25	50	50	25	53
41°	1	75	50	50	50	50	75	......	......	58
	2	100	100	100	100	75	75	......	......	92
	3	75	50	50	75	50	50	......	......	58
	4	50	50	25	50	25	50	......	......	42
	5	50	25	25	50	25	25	......	......	33
	6	00	00	00	25	00	00	......	......	4
	7	00	00	00	00	00	00	......	......	0
42°	1	25	25	25	25	50	50	......	......	33
	2	50	50	50	50	25	50	......	......	46
	3	50	25	25	25	25	25	......	......	29
	4	50	25	25	25	00	25	......	......	25
	5	25	25	25	00	25	25	......	......	21
	6	25	00	00	25	25	00	......	......	13
	7	00	00	00	00	00	00	......	......	00
43°	1	25	50	50	50	25	25	......	......	38
	2	00	50	00	00	00	00	......	......	8
	3	25	00	25	25	00	25	......	......	17
	4	00	25	00	00	25	00	......	......	8
	5	25	00	00	00	00	00	......	......	4
	6	00	00	00	00	00	00	......	......	00
	7	00	00	00	00	00	00	......	......	00

period of exposure had been 3, 6, 9, etc., hours, and the average hourly rate for each plant, for each of these longer periods, has been determined. The results thus obtained for any given time period are those that would have been exhibited if the plants had not been measured excepting at the beginning and end of that period, as though the experiment had been discontinued after 3, 6, 9, etc., hours. Table VII presents samples of these detailed results, for temperatures of 12° to 14°, 20°, 21°, 31° to 34°, and 40° to 43°C. In the

TALBE VI

Average growth increments (hundredths of a millimeter) for shoots of maize seedlings, at constant temperatures of from 12° to 43°C., for consecutive observation periods; the temperature maintained constant throughout the entire period of exposure, in each case.

TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT
12°	6	1 (3)[a]	46 (15)[b]
	6	2 (3)	8 (3)
	6	3 (3)	29 (10)
	6	4 (3)	25 (8)
13°	6	1 (3)[a]	42 (14)[b]
	6	2 (3)	17 (6)
	6	3 (3)	25 (8)
	6	4 (3)	33 (11)
	6	5 (3)	46 (15)
	6	6 (8)	129 (18)
	6	7 (2)	38 (19)
	6	8 (3)	31 (10)
	6	9 (3)	54 (18)
	6	10 (4)	65 (16)
14°	6	1 (3)[a]	58 (20)[b]
	6	2 (3)	29 (10)
	6	3 (3)	55 (18)
	6	4 (3)	55 (18)
	6	5 (3)	63 (21)
	6	6 (8)	175 (22)
	6	7 (3)	75 (25)
	6	8 (3)	79 (26)
	6	9 (3)	75 (25)
	6	10 (3)	94 (31)
15°	12	1 (3)[a]	63 (21)[b]
	12	2 (3)	38 (13)
	12	3 (3)	69 (23)
	12	4 (3)	73 (24)
	12	5 (3)	88 (29)
	6	6 (1)	21
	6	7 (2)	88[c] (29)
	6	8 (7)	242 (35)
	6	9 (3)	104 (35)
	5	10 (3)	120 (40)
	6	11 (3)	105 (35)
18°	4	1 (3)	54 (18)
	4	2 (3)	81 (27)
18°	4	3 (3)	100 (33)
	4	4 (3)	106 (35)
20°	12	1	27
	12	2	31
	12	3	38
	12	4	40
	12	5	38
	12	6	46
	12	7	48
	12	8	48
	12	9	48
	12	10	60
	12	11	56
	12	12	58
	6	13	79
	6	14	50
	6	15	50
21°	4	1	25
	4	2	38
	4	3	50
	4	4	50
	4	5	50
	4	6	56
	4	7	56
	4	8	62
	4	9	50
	4	10	62
	4	11	69
	4	12	62
22°	4	1	31
	4	2	38
	4	3	44
	4	4	44
	4	5	38
	4	6	44
	4	7	63
	4	8	75
	4	9	88

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[a] In all cases where the length of the observation period is other than one hour, its length (in hours) follows its serial number, in parenthesis.

[b] Growth increments for observation periods of more than one hour are followed by the calculated hourly rate, in parenthesis.

[c] For periods 6 and 7 together, a 3-hour period.

TABLE VI—Continued

TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT
22°	4	10	81
	4	11	88
	4	12	88
	4	13	81
23°	12	1	38
	12	2	48
	12	3	48
	12	4	63
	12	5	56
	12	6	60
	12	7	60
	12	8	85
	12	9	73
	12	10	81
	12	11	75
	12	12	83
24°	11	1	41
	11	2	52
	11	3	57
	11	4	61
	11	5	61
	11	6	77
	11	7	70
	11	8	79
	11	9	70
	11	10	82
	11	11	73
	11	12	100
25°	12	1	38
	12	2	65
	12	3	79
	12	4	71
	12	5	71
	12	6	77
	12	7	75
	12	8	75
	12	9	77
	12	10	81
	12	11	94
	12	12	102
26°	15	1	50
	15	2	67
	15	3	62
26°	15	4	75
	15	5	75
	15	6	95
	15	7	87
	15	8	88
	15	9	92
	15	10	105
	15	11	93
	15	12	90
27°	10	1	65
	10	2	73
	10	3	78
	10	4	85
	10	5	88
	10	6	85
	10	7	98
	10	8	95
	10	9	98
	10	10	108
	10	11	108
	10	12	110
28°	24	1	55
	24	2	76
	24	3	85
	24	4	92
	24	5	92
	24	6	94
	24	7	98
	24	8	111
	24	9	109
	24	10	127
	24	11	115
	24	12	127
29°	12	1	65
	12	2	75
	12	3	81
	12	4	83
	12	5	96
	12	6	88
	12	7	100
	12	8	96
	12	9	104
	12	10	119
	12	11	121
	12	12	125

TABLE VI—Continued

TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT
30°	9	1	81
	9	2	86
	9	3	100
	9	4	100
	9	5	97
	9	6	103
	9	7	117
	9	8	103
	9	9	108
	9	10	108
	9	11	144
	9	12	131
31°	6	1	54
	6	2	58
	6	3	96
	6	4	104
	6	5	133
	6	6	123
	6	7	125
	6	8	113
	6	9	125
	6	10	129
	6	11	117
	6	12	154
	6	13	129
	6	14	138
	6	15	138
	6	16	121
	6	17	129
	6	18	150
	6	19	142
	6	20	158
	6	21	138
	6	22	142
	6	23	142
	6	24	138
	6	25	142
	6	26	158
	6	27	167
	6	28	163
	6	29	158
	6	30	158
	6	31	146
31°	6	32	142
	6	33	146
	6	34	146
	6	35	154
	6	36	154
	6	37 (3)[a]	413 (138)[b]
32°	6	1	71
	6	2	75
	6	3	83
	6	4	100
	6	5	104
	6	6	121
	6	7	113
	6	8	125
	6	9	121
	6	10	125
	6	11	150
	6	12	142
	6	13	138
	6	14	142
	6	15	133
	6	16	146
	6	17	146
	6	18	142
	6	19	133
	6	20	138
	6	21	142
	6	22 (3)[a]	433 (144)[b]
	6	23 (3)	429 (143)
	6	24 (3)	404 (135)
	6	25 (3)	417 (139)
	6	26 (3)	421 (140)
	6	27 (3)	338 (113)
33°	12	1	58
	12	2	63
	12	3	83
	12	4	88
	12	5	94
	12	6	113
	12	7	117
	12	8	110
	12	9	127

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[a] In all cases where the length of the observation period is other than one hour, its length (in hours) follows its serial number, in parenthesis.

[b] Growth increments for observation periods of more than one hour are followed by the calculated hourly rate, in parenthesis.

TABLE VI—Continued

TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT
33°	12	10	119
	12	11	110
	12	12	129
	12	13	131
	12	14	129
	12	15	142
	12	16	129
	12	17	140
	12	18	110
	12	19	142
	12	20	131
	12	21	119
34°	12	1	58
	12	2	48
	12	3	71
	12	4	83
	12	5	100
	12	6	100
	12	7	125
	12	8	113
	12	9	113
	12	10	125
	12	11	121
	12	12	108
	12	13	115
	12	14	104
	12	15	113
	12	16	133
	12	17	127
	12	18	108
	12	19	144
	12	20	129
	12	21	94
35°	10	1	43
	10	2	50
	10	3	78
	10	4	85
	10	5	93
	10	6	95
	10	7	120
	10	8	83
	10	9	105
	10	10	85
	10	11	103
	10	12	90
35°	10	13	95
	10	14	108
	10	15	110
	10	16	100
	10	17	105
	10	18	100
	10	19	85
	10	20	78
	10	21	95
36°	6	1	29
	6	2	54
	6	3	67
	6	4	63
	6	5	75
	6	6	88
	6	7	83
	6	8	92
	6	9	104
	4	10	100
	4	11	75
	4	12	75
	4	13	75
	4	14	94
	4	15	119
	4	16	63
	4	17	75
	4	18	62
	4	19	69
	4	20	62
	6	21	50
	6	22 (3)[a]	188 (63)[b]
	6	23 (3)	154 (51)
	6	24 (4)	150 (38)
	6	25 (2)	8 (4)
37°	10	1	55
	10	2	48
	10	3	60
	10	4	78
	10	5	70
	10	6	75
	10	7	60
	10	8	68
	9	9	72
	9	10	69
	10	11	73

TABLE VI—Continued

TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT	TEMPERATURE, C.	NO. OF PLANTS USED	SERIAL NO. OF OBSERVATION PERIOD	AVERAGE GROWTH INCREMENT
37°	10	12	60	39°	12	4 (3)	140 (47)
	10	13	78		12	5 (3)	127 (42)
	10	14	70		12	6 (3)	127 (42)
	10	15	58		12	7 (3)	100 (33)
	4	16	94				
	4	17	88	40°	8	1 (3)[a]	81 (27)[b]
	4	18	56		8	2 (3)	128 (43)
	4	19	88		8	3 (3)	103 (34)
	4	20	69		8	4 (3)	66 (22)
	4	21	56		8	5 (3)	78 (29)
					8	6 (3)	63 (21)
38°	8	1	25		8	7 (3)	53 (18)
	8	2	38				
	8	3	31	41°	6	1 (3)[a]	58 (19)[b]
	8	4	56		6	2 (3)	92 (31)
	8	5	63		6	3 (3)	58 (19)
	8	6	63		6	4 (3)	42 (14)
	8	7	75		6	5 (3)	33 (11)
	8	8	63		6	6 (3)	4 (1)
	8	9	84		6	7 (3)	0 (0)
	8	10	66				
	8	11	59	42°	6	1 (3)[a]	33 (11)[b]
	8	12	69		6	2 (3)	46 (15)
	8	13	56		6	3 (3)	29 (10)
	8	14	53		6	4 (3)	25 (8)
	8	15	59		6	5 (3)	21 (7)
	8	16	69		6	6 (3)	13 (4)
	8	17	47		6	7 (3)	00 (0)
	8	18	44				
	4	19	56	43°	6	1 (3)[a]	38 (13)[b]
	4	20	50		6	2 (3)	8 (3)
	8	21	50		6	3 (3)	17 (6)
	8	22	34		6	4 (3)	8 (3)
					6	5 (3)	4 (1)
39°	12	1 (3)[a]	88 (29)[b]		6	6 (3)	0 (0)
	12	2 (3)	160 (53)		6	7 (3)	0 (0)
	12	3 (3)	181 (60)				

[a] In all cases where the length of the observation period is other than one hour, its length (in hours) follows its serial number, in parenthesis.

[b] Growth increments for observation periods of more than one hour are followed by the calculated hourly rate, in parenthesis.

last column of this table are given the mean hourly rates of growth for all plants employed with each temperature.

Table VIII sets forth the means for all cases dealt with, including those from the last column of table VII.

TABLE VII

Hourly rates (hundredths of a millimeter) of growth of shoots of maize seedlings, for periods of 3, 6, 9, etc. hours, at temperatures of 12°–14°, 20°, 21°, 31°–34°, and 40°–43° C.; the temperature maintained constant during the entire period in each case.

TEMPERATURE, C.	LENGTH OF PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	MEAN
	Hours													
12°	3	17	8	8	25	17	17	.....	.....	.....	.....	.....	.....	15
	6	8	8	8	13	8	8	.....	.....	.....	.....	.....	.....	9
	9	8	8	6	11	11	11	.....	.....	.....	.....	.....	.....	9
	12	6	8	6	8	8	17	.....	.....	.....	.....	.....	.....	9
13°	3	8	17	8	17	17	17	.....	.....	.....	.....	.....	.....	14
	6	8	8	8	13	13	8	.....	.....	.....	.....	.....	.....	10
	9	8	8	8	11	11	8	.....	.....	.....	.....	.....	.....	10
	12	8	8	8	10	15	8	.....	.....	.....	.....	.....	.....	10
	15	10	10	8	12	15	10	.....	.....	.....	.....	.....	.....	11
	23	11	13	10	14	18	10	.....	.....	.....	.....	.....	.....	13
	28	13	13	11	13	17	10	.....	.....	.....	.....	.....	.....	13
	31	14	13	10	14	18	10	.....	.....	.....	.....	.....	.....	13
	35	14	.....	11	14	19	10	.....	.....	.....	.....	.....	.....	14
14°	3	17	17	17	17	17	33	.....	.....	.....	.....	.....	.....	20
	6	13	13	13	13	13	25	.....	.....	.....	.....	.....	.....	15
	9	14	17	14	.....	14	22	.....	.....	.....	.....	.....	.....	16
	12	15	17	15	13	15	23	.....	.....	.....	.....	.....	.....	16
	15	17	18	15	13	15	23	.....	.....	.....	.....	.....	.....	17
	23	20	21	18	12	16	25	.....	.....	.....	.....	.....	.....	19
	27	19	20	19	13	17	24	.....	.....	.....	.....	.....	.....	19
	30	20	22	19	13	18	23	.....	.....	.....	.....	.....	.....	19
	33	19	22	.....	.....	19	24	.....	.....	.....	.....	.....	.....	21
	36	19	23	23	13	20	25	.....	.....	.....	.....	.....	.....	21
15°	3	17	17	17	25	50	42	8	17	17	17	8	17	21
	6	13	13	21	17	29	25	8	13	13	13	17	25	17
	9	14	19	22	22	28	25	14	14	14	19	14	19	19
	12	17	21	25	23	25	25	15	17	17	23	15	21	20
	15	18	22	25	25	25	25	18	20	20	25	17	23	22
	18	.....	.....	.....	.....	.....	.....	21	21	22	26	19	22	22
	25	.....	.....	.....	.....	.....	.....	23	23	23	33	24	27	26
	28	.....	.....	.....	.....	.....	.....	24	24	24	35	24	28	27
	31	.....	.....	.....	.....	.....	.....	24	25	25	37	27	.....	28
	34	.....	.....	.....	.....	.....	.....	25	26	26	38	26	28	28
20°	3	33	25	33	25	42	25	42	42	42	25	33	17	32
	6	42	33	42	29	37	25	42	42	46	33	33	33	36
	9	47	39	50	33	36	28	44	42	50	39	36	44	41
	12	52	44	54	38	39	31	48	46	54	44	42	50	45
	15	62	48	58	42	42	33	.....	.....	.....	.....	.....	.....	48

TABLE VII—Continued

TEMPERATURE, C.	LENGTH OF PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	MEAN
	Hours													
21°	3	33	42	25	50	...	...	...	...	...	...	...	...	38
	6	42	46	42	50	...	...	...	...	...	...	...	...	45
	9	47	47	50	50	...	...	...	...	...	...	...	...	49
	12	54	54	52	50	...	...	...	...	...	...	...	...	53
31°	3	41	66	91	75	83	58	...	...	...	...	...	...	69
	6	83	83	96	108	100	87	...	...	...	...	...	...	93
	9	100	88	92	119	102	100	...	...	...	...	...	...	100
	12	112	91	106	133	108	108	...	...	...	...	...	...	109
	15	120	97	107	145	110	117	...	...	...	...	...	...	116
	18	119	100	108	148	111	120	...	...	...	...	...	...	117
	21	121	105	113	151	113	123	...	...	...	...	...	...	121
	24	123	110	115	153	114	125	...	...	...	...	...	...	123
	27	127	114	120	154	118	126	...	...	...	...	...	...	126
	30	130	116	128	156	120	130	...	...	...	...	...	...	130
	33	132	118	131	158	120	131	...	...	...	...	...	...	131
	36	133	121	133	158	121	133	...	...	...	...	...	...	133
	39	133	123	132	157	121	134	...	...	...	...	...	...	133
32°	3	75	75	83	75	66	75	...	...	...	...	...	...	72
	6	100	87	91	91	79	104	...	...	...	...	...	...	92
	9	106	97	100	100	86	119	...	...	...	...	...	...	101
	12	111	108	110	104	104	127	...	...	...	...	...	...	111
	15	115	117	116	110	110	128	...	...	...	...	...	...	116
	18	117	119	122	118	119	129	...	...	...	...	...	...	120
	21	119	121	123	124	122	129	...	...	...	...	...	...	123
	24	123	123	126	127	125	131	...	...	...	...	...	...	125
	27	126	126	128	128	126	132	...	...	...	...	...	...	127
	30	127	127	128	129	127	133	...	...	...	...	...	...	128
	33	127	127	129	129	129	134	...	...	...	...	...	...	129
	36	129	130	129	131	128	133	...	...	...	...	...	...	130
	39	132	130	125	129	125	132	...	...	...	...	...	...	128
33°	3	75	83	75	58	81	66	50	50	66	75	83	50	68
	6	83	87	91	83	91	91	71	75	83	79	91	71	83
	9	94	91	97	94	105	102	86	86	94	88	108	86	94
	12	98	100	104	96	108	110	91	96	108	92	116	94	101
	15	100	103	116	98	110	115	95	105	113	100	133	103	107
	18	101	107	125	101	114	116	97	109	116	100	134	108	110
	21	102	108	130	105	118	118	100	112	118	102	138	112	113
34°	3	41	41	58	41	75	66	41	100	75	58	50	58	59
	6	66	75	79	42	96	83	58	100	95	83	66	75	76
	9	80	86	86	52	108	94	72	122	114	102	72	88	89
	12	89	100	92	62	110	102	75	133	119	108	77	94	97

TABLE VII—Continued

TEMPERATURE, C.	LENGTH OF PERIOD	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	MEAN
	Hours													
34°	15	90	106	93	68	115	106	80	135	120	111	77	93	100
	18	97	114	91	76	120	109	83	137	122	118	79	93	103
	21	103	119	92	83	122	113	88	134	122	122	80	91	106
40°	3	33	25	25	25	25	33	25	25	...	...	...	...	27
	6	37	37	33	41	33	37	29	29	...	...	...	...	34
	9	33	36	36	39	33	36	31	30	...	...	...	...	34
	12	31	35	31	31	29	35	29	29	...	...	...	...	31
	15	31	35	30	31	28	32	28	26	...	...	...	...	30
	18	30	33	29	29	26	29	26	25	...	...	...	...	28
	21	30	32	27	27	23	27	25	24	...	...	...	...	26
41°	3	25	16	16	16	16	25	...	...	...	...	...	...	19
	6	29	25	25	25	21	25	...	...	...	...	...	...	25
	9	27	22	22	25	19	22	...	...	...	...	...	...	23
	12	25	20	19	23	16	20	...	...	...	...	...	...	20
	15	23	18	16	20	15	18	...	...	...	...	...	...	18
	18	19	15	12	13	12	15	...	...	...	...	...	...	14
	21	16[a]	13	10	11	10	13	...	...	...	...	...	...	12[a]
42°	3	8	8	8	8	8	16	...	...	...	...	...	...	9
	6	13	13	12	13	12	16	...	...	...	...	...	...	13
	9	14	11	11	11	11	14	...	...	...	...	...	...	12
	12	14	10	10	10	8	12	...	...	...	...	...	...	11
	15	13	10	10	8	8	12	...	...	...	...	...	...	10
	18	13	8	8	9	8	10	...	...	...	...	...	...	9
	21	11[a]	7	7	8	7	9	...	...	...	...	...	...	8[a]
43°	3	8	16	16	16	8	8	...	...	...	...	...	...	12
	6	4	16	16	8	4	4	...	...	...	...	...	...	9
	9	5	11	8	8	3	5	...	...	...	...	...	...	7
	12	4	10	6	6	4	4	...	...	...	...	...	...	6
	15	5	10	5	5	3	3	...	...	...	...	...	...	5
	18	4[a]	8	4	4	3	3	...	...	...	...	...	...	4[a]

[a] There was no growth at all during the last 3 hours, so that the hourly rates for the longest period here begin a regular decrease, which would continue indefinitely, assuming that growth does not again begin. In all these cases observations were continued through the 21-hour period.

An examination of the data given in tables VII and VIII brings out clearly enough the logical futility of any consideration of an optimum temperature for growth without at the same time considering the length of the exposure period.

With temperatures of from 12° to 15°, the mean hourly rate for the first 3 hours of exposure is much higher than it is for periods of 6 and 9 hours.

TABLE VIII

Mean average hourly growth rates (hundredths of a millimeter), for shoots of maize seedlings, at temperatures of from 12° to 43°, for observation periods of 3, 6, 9, etc., hours, the temperature maintained constant, in each case, throughout the entire period.

TEMP.	LENGTH OF EXPOSURE PERIOD, *hours*												
	3	6	9	12	15	18	21	24	27	30	33	36	39
deg. C.													
12	15	9	9	9	......	......	......	......	......	......	......	......	......
13	14	10	10	10	11	......	......	13 (23)[a]	13 (28)	13 (31)	......	14 (35)	......
14	20	15	16	16	17	......	......	19 (23)	19	19	21	21	......
15	21	17	19	20	22	22	......	26 (25)	27 (28)	28 (31)	28 (34)	......	......
18	18	23	26	28	......	......	......	......	......	......	......	......	......
20	32	36	41	45	48	......	......	......	......	......	......	......	......
21	37	44	48	53	......	......	......	......	......	......	......	......	......
22	37	39	51	59	......	......	......	......	......	......	......	......	......
23	44	52	59	64	......	......	......	......	......	......	......	......	......
24	50	58	64	69	......	......	......	......	......	......	......	......	......
25	60	66	70	75	......	......	......	......	......	......	......	......	......
26	58	65	75	82	......	......	......	......	......	......	......	......	......
27	70	78	83	90	......	......	......	......	......	......	......	......	......
28	72	80	89	98	......	......	......	......	......	......	......	......	......
29	88	89	94	105	......	......	......	......	......	......	......	......	......
30	77	94	100	108	......	......	......	......	......	......	......	......	......
31	69	93	100	109	116	117	121	123	126	130	131	133	133
32	72	92	101	111	116	120	123	125	127	128	129	**130**	128
33	68	83	94	101	107	110	113	......	......	......	......	......	......
34	59	76	89	97	100	103	106	......	......	......	......	......	......
35	56	74	84	86	89	92	92	......	......	......	......	......	......
36	47	65	72	74	**76**	**76**	73	71	......	......	......	......	......
37	55	65	69	70	**72**	70	68	......	......	......	......	......	......
38	31	46	55	**58**	57	56	53	47	......	......	......	......	......
39	31	41	**47**	46	46	45	43	......	......	......	......	......	......
40	27	**34**	**34**	31	30	28	26	......	......	......	......	......	......
41	19	**25**	23	20	18	14	(12)[b]	......	......	......	......	......	......
42	9	**13**	12	11	10	9	(8)[b]	......	......	......	......	......	......
43	**12**	9	7	6	5	(4)[b]	......	......	......	......	......	......	......

[a] Where the length of exposure period is not that given at the head of the column, the actual length is shown in parenthesis, after the corresponding rate.

[b] The last rate given for 41°, 42° and 43°, in parenthesis, is based on a period of exposure continuing 3 hours after all growth had ceased; it is, of course, a false average, in one sense.

These exceptionally high rates for the first 3 hours may be due to after-effects of the higher temperature to which the plants had been exposed previous to the beginning of the experiments. With longer periods, however, the high rate for the first 3 hours is equalled or surpassed, at least for temperatures of 13° to 15°. Whether periods still longer than 35 hours (for 13°), 36 hours (for 14°) or 34 hours (for 15°) might have given still higher mean hourly rates than the highest ones shown can, of course, not be stated.

Whatever may be the explanation of the high hourly means for the first 3 hours, it is clear that the means generally increase with longer periods, at least up to 34 to 36 hours.

For temperatures 20° to 30°, the mean hourly rates for each temperature also become generally of greater magnitude as the exposure period becomes longer. For temperatures 31° and 32°, where observation was longest continued, this statement still holds, but for 32° a maximum hourly mean (130) is encountered with a period of 36 hours, after which the magnitude of the mean apparently declines; the mean rate during the 39-hour period (128) is as low as that for the 30-hour period.

For temperatures 33° to 35°, as here given, the observations were discontinued too soon to show such a decrease in hourly rate as that just mentioned, if such decrease occurs. A special series at 33.5°, continued 36 hours (not represented in the tables), shows this, however; it gives the highest hourly rates (111 in each case) with exposure periods 30 and 33 hours long.

The decrease in question is clearly shown for temperatures 36° to 42°. For 35°, the 18 and 21-hour periods give the highest mean hourly rate (92), though the data do not show that a still longer period might not have exhibited a greater value. From 36° to 43°, however, the length of exposure period resulting in the highest mean rate becomes progressively shorter with higher temperature. These maximum means are indicated in table VIII by full-face type, and it is clearly seen that the positions of these numbers in the table picture a very regular curve.

It has been shown, then: (1) that in all cases, from 13° to 42°, the mean hourly rate of growth increases with periods of exposure of more than 3 hours; (2) that, with temperatures of 32°, 33.5°, and 36° to 42°, this increase reaches a maximum and is then followed by a decrease, as the periods become longer; and (3) that for temperatures above 31°, the maximum mean hourly rate is attained for shorter and shorter periods of exposure, as the temperature becomes higher. For 43°, it appears that a period of 3 hours gives a higher hourly rate than one of 6 or more hours; in other words, the maximum in question is attained within the 3-hour period, and is followed, as in the other cases, by a decrease.

DISCUSSION

The optimum temperature for growth. As has been emphasized, the term optimum temperature cannot be employed in the common, loose sense, as though there were a definite optimum for each plant. No definition of optimum temperature can be valuable unless it involves a consideration of time.

If we regard as the optimum "the highest temperature which can be permanently sustained without depression of function" (F. F. Blackman [05]), then it is clear that such an optimum cannot exist, for there is *no* temperature at which any given growth rate will be indefinitely maintained. Of course the optimum temperature for growth might be defined as the highest one which will allow growth *for some specified time period*, without decrease in rate, but here the condition of time is involved.

Again, if we consider, also with F. F. Blackman [05], that the optimum is that temperature for which "the retardation produced by exposure to superoptimal temperature must not be of the nature of permanent injury, and that therefore on cooling again to the optimum temperature there must be a return of the function to its highest value," it is obvious that the length of the period of exposure to "superoptimal temperature" must be specified. Otherwise there can be no such optimum.

It seems most useful to regard as the optimum that temperature which *for a specified period of exposure*, produces the greatest growth. On such a basis several optima are shown in table VIII.

Referring to that table, if the experiments had been made with a 3-hour period the optimum temperature deduced would have been 29°, with an average hourly rate of 88. If measurements had been made only at the beginning and end of the first 6 hours of exposure, the indicated optimum would have been at 30°, with an hourly growth rate of 94. The optimum temperature for 9 and for 12 hours of growth is clearly 32°. For longer periods the data at hand are not as clear, but it appears that a temperature of 32° is the optimum for 18 and 21, probably also for 24 and 27 hours, and that for longer periods (as far as shown) the optimum appears to shift to 31°. Whether the last observation is important cannot now be determined, but the consistency of the available data argues against this being due to uncontrolled conditions. Also, whether the optimum may shift over a range still somewhat greater than that from 29° (or below) to 32°, cannot be decided without longer continued series of observations. It seems to be clear, however that, *for periods of 3 hours or longer*, the temperature giving the greatest mean hourly rate of growth lies within the temperature range 29°–32°.

To bring out these relations in the usual manner growth curves have been constructed for the mean growth rates for periods of 3, 6, 9, 12, 15 and 21 hours as given in table VIII, and these curves are given in figures 2 and 3. They may be compared to the curve of figure 1, based on Koeppen's measurements for a period of 48 hours. In these curves the abscissae represent the temperature and the ordinates the mean hourly rate of growth, in hundredths of a millimeter.

It will be at once noted that there is not, in any of the curves of figures 2 and 3, any indication of such a double optimum temperature for growth, as is shown in Koeppen's curve. The most interesting feature of my curves lies in the shifting of the curve maximum already emphasized. Of course this shifting may be related to the same causes as those that produced Koeppen's double maximum, which, he ventured to suggest, might have been due to "eine chemische Verbindung in einer solchen Weise, dass dadurch das Wachsthum eine Verzögerung erleidet." But, if such be the case, there is here no indication of it. Neither this suggestion nor that of MacDougal[21] in the same connection (to the effect that the first pronounced drop in Koeppen's curve might perhaps be due to phase changes of cell colloids and the second to the inhibition of specific enzyme action), requires *a priori* discussion here. I am of the opinion that the very pronounced irregularities in Koeppen's curve, the two greatest of which produce the apparent double optimum, are probably to be attributed to the small number of seedlings used by Koeppen, to features of his culture methods, and to his failure to select uniformly growing plantlets at the outset of each experiment. In short, these irregularities appear to have been due to unknown and uncontrolled conditions.

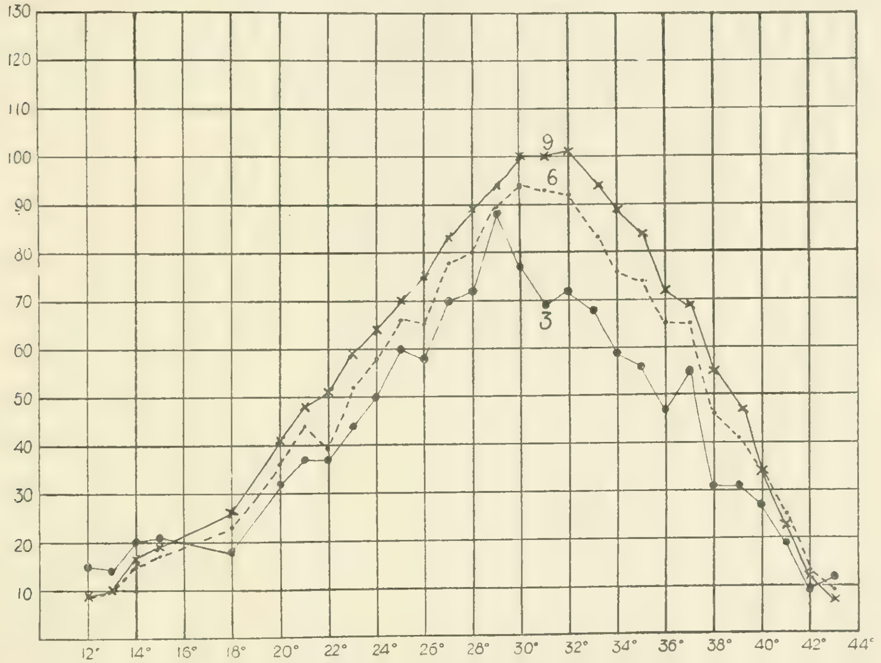

FIG. 2. Graphs showing the relation of temperature to average hourly growth rate, in shoots of maize seedlings, for 3, 6 and 9 hours, respectively. The variation in the optimum temperature, with different time periods, is shown. Ordinates are in terms of hundredths of a millimeter.

Aside from the irregularities of Koeppen's curve, my own agree with it in general form, excepting that the slopes on either side of the curve maximum are never nearly so steep as in Koeppen's. My curves for periods above 3 hours all agree in showing a rounded or flattened apex, in which respect they also agree with the form that Koeppen's curve would assume if it were conventionalized in the usual way, suppressing the marked irregularities; the much discussed double optimum appears to be merely an indication of a rounded apex, denoting that a considerable temperature change in this region has but little effect upon the mean hourly rate of growth.

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[21] MacDougal, D. T., The auxo-thermal integration of climatic complexes. Amer. Jour. Bot. 1: 186–93. 1914.

Relation of the growth rate to van't Hoff's law. The principle of chemical reaction velocity, to the effect that this velocity is doubled or trebled with each rise in temperature of 10°C., frequently termed the van't Hoff law,[22] has been applied to physiological processes by numerous writers.[23] In dealing with this matter it is customary to employ the term *temperature coefficient* to denote the factor by which the process velocity in question is affected for each change in temperature of 10°C. This coefficient remains approximately constant within a certain range of temperature changes, beyond which its value alters markedly.

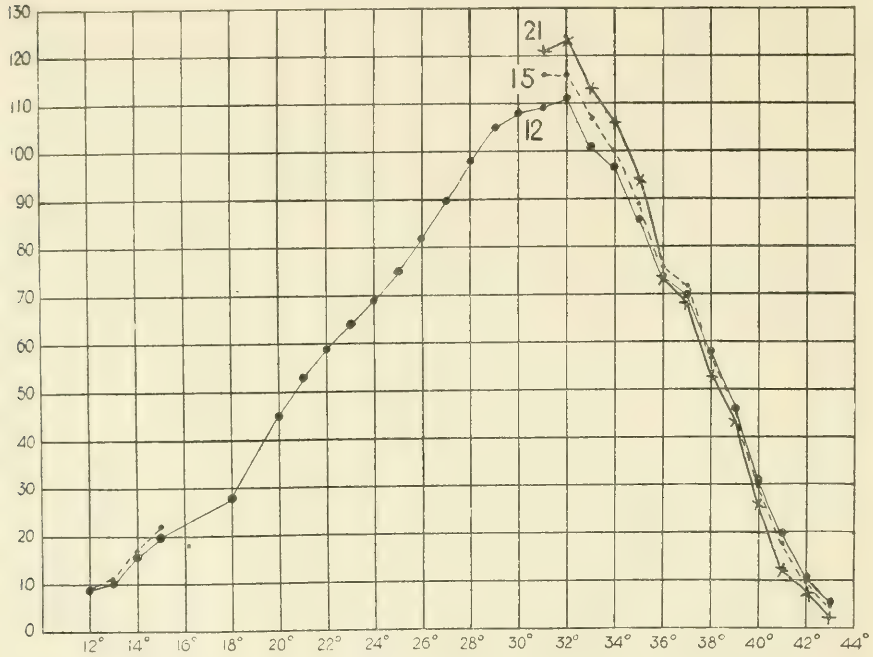

FIG. 3. Graphs showing the relation of temperature to average hourly growth rate, in shoots of maize seedlings, for 12, 15 and 21 hours, respectively. Ordinates are in terms of hundredths of a millimeter.

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[22] Van't Hoff, J. H., Lectures on theoretical and physical chemistry, translated by R. A. Lehfeldt. London. No date (author's preface dated 1898). Part I, page 227.

[23] A rather full list of references to this discussion are given in the following:—Livingston, B. E., and Livingston, G. J., Temperature coefficients in plant geography and climatology. Bot. Gaz. **56**: 349–75. 1913.

For the process of respiration in lupine seedlings and Syringa flowers, Clausen [90] found the temperature coefficient to have a value of 2.5 for a range from 0° to 20°C. Matthaei [04] showed that the rate of photosynthesis in leaves of cherry-laurel is affected by a temperature coefficient of 2.1, for a range from 9° to 19°C. For sunflower leaves she found the corresponding coefficient to be 2.3. Balls[24] showed the van't Hoff law to hold for the process of growth in the "sore-shin" fungus, and Herzog[25] found the same to be true for spore-formation in *Saccharomyces pasteurianus.* Price [11] concluded that, "on the whole, an increase in temperature of 10°C. for a given kind of bud [of deciduous fruit trees] lessens the time of blooming by about one-half" (page 210). The same author shows a photograph of maize seedlings grown one week at 21°, 26° and 31°C., respectively, which appears to exhibit a coefficient of nearly 4 for this temperature increase of 10°, but he fails to refer to this matter in the text. In general, it appears that many or most physiological processes follow the van't Hoff principle within a medium range of temperatures, having temperature coefficients of from about 2 to about 2.5.

TABLE IX

Temperature coefficients for growth rate of shoots of maize seedlings, for various 10° ranges of temperature, the time period being 12 hours.

TEMPERATURE RANGE	RANGE OF GROWTH RATE	COEFFICIENT
deg. C.	*0.01 mm.*	
12–22	9– 59	6.56
13–23	10– 64	6.40
15–25	20– 75	3.75
18–28	28– 98	3.50
20–30	45–108	2.40
21–31	53–109	2.06
22–32	59–111	1.88
25–35	75– 86	1.15
32–42	111– 11	0.09
33–43	101– 6	0.06

The growth measurements of the present study furnish data for determining within what limits this process, in shoots of maize seedlings, may follow the chemical principle. Considering only the mean hourly growth rates for a period of 12 hours, table IX gives the temperature coefficients effective for various 10° variations in temperature.

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[24] Balls, W. L., Temperature and growth. Ann. Bot. **22**: 557–91. 1908.

[25] Herzog, R., Zur Biologie der Hefe. Zeitschr. physiol. Chem. **37**: 396–9. 1903.

Of course the coefficients of table IX would necessarily alter to some extent in their relations if any other time period than 12 hours were taken as basis for the growth rates. The table shows that the coefficient varies from 2.40 to 1.88 with the temperature range 20° to 32°. The latter range is but a small portion of the total temperature range within which growth of maize seedlings is shown to occur, but it is interesting to note that 20° to 32° represents the order of the temperature range usually experienced in maize-growing regions during the days when the plantlets are at a stage of growth comparable to that with which this study deals.

As in the case of most chemical reactions, the magnitude of the coefficient decreases as the temperature increases; and, conversely, this magnitude is simply approximately constant for a limited range of medium temperatures.

On the whole, the van't Hoff principle appears to apply to the growth process in maize seedlings and variation in the temperature coefficient is confined to a range of about 1.88 to 2.40, for the temperature range 20° to 32°C.

Minimum temperature for growth. The data here set forth show that the minimum and the maximum, like the optimum, temperature for growth must be considered as conditioned by the length of the period of exposure as well as by the temperature itself.

The minimum must needs be defined as the temperature at which growth ceases *after a specified exposure period.* The lowest temperature here dealt with (12°) is clearly still well above the minimum for an exposure period of 12 hours or less; growth, although slow and irregular, was not apparently brought to a standstill during the 12-hour experiments at 12°.

As to whether the hourly growth rate at low temperatures falls or rises, or remains constant, with increasing length of exposure period, this matter has attracted attention in the literature and may be given brief consideration here. Kirchner[26] found that low temperatures retarded plant growth often a longer or shorter period of time. He observed "ein allmähliches Sinken der Zuwachse innerhalb gleicher Zeiträume, welches in manchen Fällen erst nach einigen Wochen, in andern schon nach einem bis wenigen Tagen mit völligen Wachsthums-Stillstande, resp. mit dem Turgorverlust der Wurzel endigt."

Askenasy [90] obtained very different results from a similar inquiry, concluding that there is no such decrease in growth rate as Kirchner found. He suggests that this disagreement is due to improper treatment of Kirchner's seedlings. Askenasy finds, in his own results, "durchaus keinen Anhalt dafür, dass bei niederer Temperatur ein constantes Fallen der Zuwachsgrössen statt findet . . . . ."

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[26] Kirchner, O., Ueber das Längenwachsthum von Pflanzenorganen bei niederen Temperaturen. Biol. Pflanzen 3: 335–64. 1883.

In my own experiments (table VI) there occurred a fall in growth rate after the first three hours at 12° to 15°C., but this fall was followed (with temperatures of 13° to 15°) by a gradual increase in the hourly rate, this increase being continued up to 35 hours, in the case of 14°, and at least to 31 hours, in that of 15°. As has been remarked, it is very difficult to work precisely at these low temperatures, on account of the low growth rates here encountered.

Maximum temperature for growth. If the maximum temperature is defined similarly to the minimum and optimum, in terms of Centigrade degrees and time of exposure, we find (table V) that a temperature of 40° still allowed growth in all the plants tested, at least for a period of 21 hours; therefore, 40° is not the maximum for time periods below 21 hours. The three still higher temperatures tested all produced cessation of growth within the period of the experiments. With a temperature of 41° all but one of 6 plants exhibited no growth after 15 hours, the remaining plant ceased to grow after 18 hours. With 42°, 3 plants ceased growth after 15 hours and 3 after 18 hours. With 43° 3 plants grew no more after 9 hours, 2 ceased to grow after 12 hours, and growth of the remaining plant was stopped after 15 hours. Finally, it may be stated that 43°C. is the maximum temperature for growth of these seedlings, for an exposure period of 15 hours; 42° is the corresponding maximum for 18 hours. For time periods shorter than 15 hours the maximum temperature was not attained in the present series, but it must obviously be higher than 43°C.

Plants kept at these high temperatures (41° to 43°) for 6 hours after growth had ceased were not killed; when the temperature was gradually lowered growth was resumed.

Alteration in mean hourly growth rate with prolonged exposure to constant temperature. It appears from the data of table VI that, for all temperatures from 13° to 42°, the actual growth rate becomes greater as time goes on. With temperatures above 30° this increased actual rate is shown to reach a maximum and then to decline, but there is considerable apparent irregularity in the time required, with the various temperatures, for the attainment of the maximum average rate of growth.

This alteration in growth rate for successive hours of the prolonged exposure period is evidenced in the similar but much more regular march of the mean hourly rates derived from longer and longer periods, as brought together in table VIII. As has been pointed out, the length of period required to give the maximum mean hourly rate of growth for a given temperature, at least from 31° upward, becomes shorter with higher temperature. Thus for 31° this mean increases with longer and longer periods, up to 36 hours, and no decrease is noted with a period of 39 hours. With 32° this value increases until the period of exposure attains a length of 36 hours, and after 39 hours a decrease is evident. This decrease is shown with a period of only 6 hours at a temperature of 43° and the points in table VIII, at which the maximum values of the mean hourly rates occur, for the temperatures from 32° to 43°, clearly arrange themselves in a very regular curve.

The general phenomenon of decreased rate with prolonged exposure to high temperature has been pointed out and discussed by a number of writers, with reference to growth as well as other processes. Thus, Sachs [75], as early as 1863, showed that Mimosa can withstand a temperature of 50°C., or even higher, for several minutes, but too long exposure produced death.

Eidam,[27] subjected Bacterium termo to a temperature of 45° to 47°C., for from 30 minutes to 3 hours, and found that this produced no ill effects; when subjected, however, to such temperature for a period longer than 4 hours, its growth subsequently, at a lower temperature, was retarded.

Ward,[28] studying cell division and growth of Bacillus ramosus in relation to temperature, says (page 446), "With high temperature cultures I never got anything like so large a crop at 30–35°C. as at 22–25°C., other things being equal. Moreover, it seems clear that though the growth may, for a short time, be as rapid as it is near the optimum, it soon slows down."

Ewart,[29] in his studies of the relation of temperature to the rate of protoplasmic streaming, found that at 30°C. a decrease in rate was manifested after a period of 20 minutes; at 45°C. there was a marked decrease of rate after but 6 minutes.

Turning our attention to more definitely chemical processes, in fermentation, according to Chudiakow [94], a decrease in rate is manifested at 25°C. after 5 hours; a more rapid decrease is shown at 35°C., in 2 hours; at 40°C. the decreased rate manifests itself from the beginning of the exposure to this temperature.

In a study of photosynthesis in relation to light, Pantanelli[30] found that with the higher light intensities a decrease in the photsynthetic values occurs after a period of time.

My own results are in general conformity with Matthaei's [04] first and third laws of the temperature control of photosynthesis at high temperatures. That writer's second law (namely, "the higher the temperature the more rapid is the rate of falling off"—see also F. F. Blackman [05], page 283) is not always so clearly shown here.

F. F. Blackman [05] has called attention to the significance of the decrease in the velocity of physiological processes occurring with prolonged exposure to high temperatures, and he has well emphasized the importance of this

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[27] Eidam, E., Die Einwirkung verschiedener Temperaturen und des Eintrocknens auf die Entwicklung von Bacterium termo (Duj.). Biol. Pflanzen **1**: 208–24. 1875.

[28] Ward, H. M., On the Biology of Bacillus ramosus (Fraenkel) a Schizomycete of the River Thames. Proc. Roy. Soc. Lond. **58**: 265–468. 1895.

[29] Ewart, A. J., On the physics and physiology of protoplasmic streaming in plants. Oxford, 1903.

[30] Pantanelli, E., Abhängigkeit der Sauerstoffausscheidung belichteter Pflanzen von äusseren Bedingungen. Jahrb. wiss. Bot. **39**: 167–228. 1904.

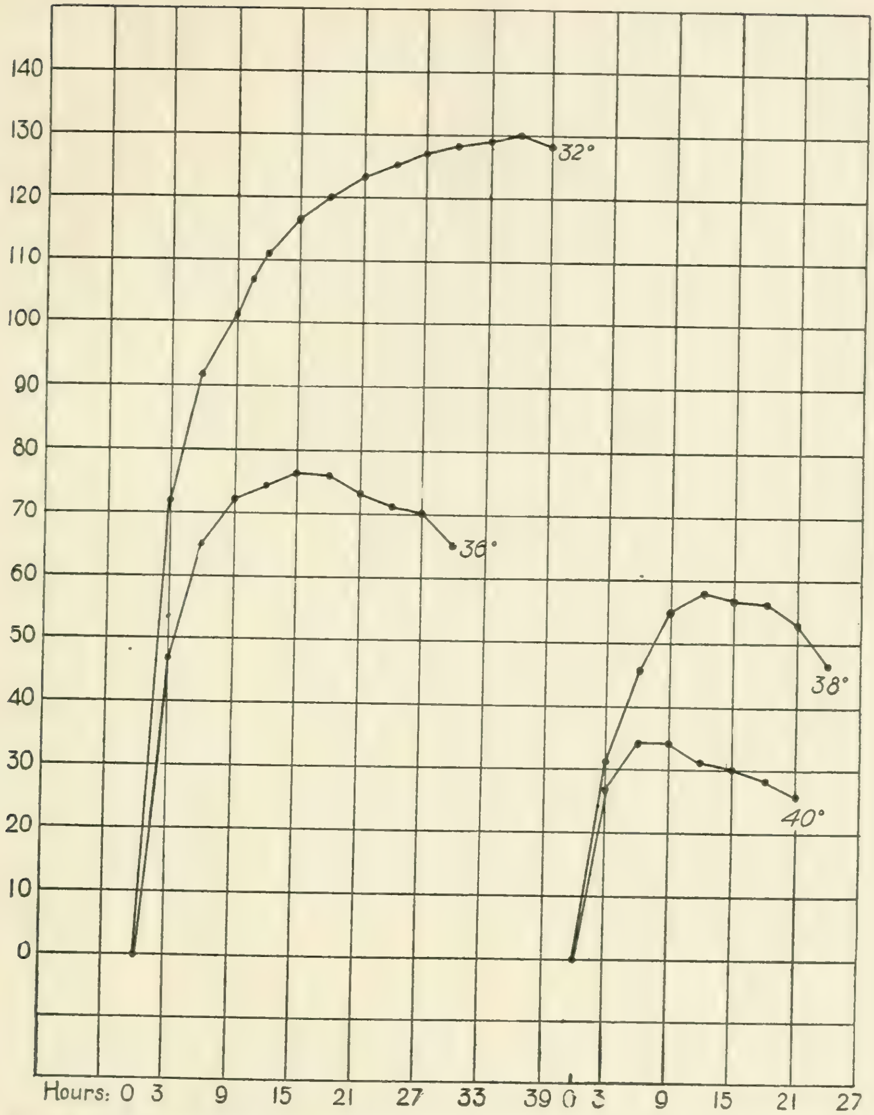

FIG. 4. Graphs showing the average hourly growth rates, in shoots of maize seedlings, for periods of 3, 6, 9, etc. hours, with maintained temperatures of 32°, 36°, 38° and 40°C. Ordinates are in terms of hundredths of a millimeter.

decrease in the determination of the position of the optimum temperature. From Matthaei's data Blackman points out that, in order to detect the maximum rate of photosynthesis, the initial measurement, especially with high temperatures, must be obtained as soon as possible after the beginning of the exposure period. My own data exhibit the decrease in hourly rate of growth as beginning after a much longer period than is the case with photosynthesis, so that, to detect the maximum rate it appears unnecessary to begin observation until after nearly 3 hours with 43° temperature, and a longer time period may safely elapse in the case of lower temperatures. The initial time period should not be longer than 39 hours for 31°.

The march of the mean hourly growth rate for maize seedlings, with increasing length of exposure period, is shown graphically in figure 4, for the temperatures 32°, 36°, 38° and 40°C. The data are taken from table VIII. The initial rise in this mean rate is very rapid but the curves become less steep as the curve maximum is approached. This kind of phenomenon also occurs with growth of *Bacillus ramosus*, according to Ward [95], who says (page 446), "at the optimum it metabolises, and grows and respires, etc., at its best; but at higher temperatures removed from that it may grow for a short time more rapidly, but soon exhausts itself, and so produces a poorer crop in the end." Also, the effects of high temperature on the velocity of enzyme action, as obtained by Tammann[31] and others, agree most beautifully with the results on growth herein recorded. In this general connection it may be mentioned that the effects of high temperatures upon certain animal processes—such phenomena as heat-depression,[32] for example—show some interesting analogies to the matters here considered.

An explanation of the regular alteration in growth rate, occurring with sufficiently prolonged exposure to high temperatures, cannot yet be even attempted in any useful way, even in the realm of pure theory. Growth is such an exceedingly complex process that the interpretation here needed must await much more complete analysis of growth relations in general than has yet been possible. Some of the external conditions of growth are fairly easy to control and our knowledge of these as limiting factors is already considerable, but of internal conditions, within the plant, we know practically nothing of a quantitative nature.

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[31] Tammann, G., Zur Wirkung ungeförmter Fermente. Zeitschr. physik. Chem. **18**: 426–42. 1895. See also Herzog [03].

[32] Winterstein, H., Ueber die Wirkung der Wärme auf den Biotonus der Nervencentren. Zeitschr. Allg. Physiol. **1**: 129–41. 1902.

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END RESULTS OF DESICCATION AND RESPIRATION IN SUCCULENT PLANTS

D. T. MACDOUGAL, E. R. LONG AND J. G. BROWN

ABSTRACT[1]

A large number of seed-plants absorb a much greater quantity of water during certain seasons or in a certain stage of their development than is lost, and the surplus may accumulate in exaggerated cortical or medullary tracts in the roots, stems or leaves. The stored water in many instances holds soluble carbohydrates, or these substances may be present in the form of starch, etc. Large water balances are especially characteristic of the succulents of semi-arid regions in which the rainfall comes within well-defined annual periods. This feature is especially illustrated by the cacti, certain forms of which were used for experimental material in the studies described in this paper.

A number of large, sound individuals of Echinocactus, and of severed joints of flat stems of Opuntia were deprived of a water supply, and compelled to carry on existence at the expense of accumulated water and food-material. Some of the preparations were exposed to the full illumination to which they were accustomed, and others were placed in diffuse light, obtaining differential effects in water-loss, respiration, disintegration of acids, and photosynthesis. The principal generalizations arising from the studies are as follows:

1. Echinocactus in diffuse light may lose as much as one two-thousandth part of its weight in one day, immediately following the excision of its root-system. The same plant six years later, under equivalent conditions except that its weight had been reduced nearly a third, lost no more than one part in seventeen thousand of its weight in one day.

2. An Echinocactus weighing 38 kg., of which 90–95 per cent may be estimated as water, lost 3.5 kg., or one-tenth of its total water, in the first year of isolation in diffuse light. In the sixth year the loss was one-twentieth of the water supply at the beginning of that year.

3. Echinocacti in the open lost 38–45 per cent of their original weight during the period from June to November inclusive. Individuals in the diffuse light of the experimental rooms lost 7 or 8 per cent in the same period.

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[1] The manuscript of this paper was received June 12, 1915. This abstract was preprinted, without change, from these types and was issued as Physiological Researches, Preliminary Abstracts, vol. 1, no. 6, August, 1915.

4. An Echinocactus in the open may survive no more than two years at the expense of its surplus food-material and water. Similar plants in diffuse light have been seen to be sound after six years of starvation, although the effects were marked.

5. Prolonged confinement in diffuse light results in a decrease in density of sap in Echinocactus. Exposure in the open, with consequent rapid loss in weight, may be followed by an increase or by a decrease in the density of the sap.

6. Decrease in the density of sap is to be attributed to a disintegration of the carbohydrates, which, in Echinocactus No. 7, amounted to 13 per cent of the dry weight of the cortex. The destruction of material was extended to include the walls of whole masses of tissue in the cortex.

7. Increase in the density of the sap might result from rapid evaporation, which altered the proportions of water and dissolved substances, or by the addition of photosynthetic products.

8. The proportion of reducing sugars is greatest in the peripheral tissues of normal plants, in connection with the photosynthetic activity localized here, and decreases through the cortex to the central cylinder. The reduction which takes place in desiccation and starvation reverses the distribution of these substances, the greatest proportion after desiccation being found in the inner cortex and the total amount being reduced.

9. Non-reducing soluble sugars, which are present in only minute proportions if at all in normal Echinocacti, are noticeable constituents of the sap of desiccated plants.

10. The acidity of the tissues is due to certain modifiable features of respiration. Acidity of plants which have undergone long continued desiccation and starvation is low, since the amount of carbohydrate from which they are derived has been decreased.

11. Catabolism in extended desiccation and starvation eventually breaks down the plasmatic colloids, and includes hydrolysis of the cell-walls of the cortex.

12. The water-absorbing capacity of cortical tissues of Echinocactus is a resultant of the osmotic activity of the solutions and of the hydratation power of the colloids, consequently great diversity was found in the various specimens examined with regard to this feature.

13. Echinocactus is capable of growth in the apical region, in plants in which water loss and disintegration of the carbohydrates (including hydrolysis of the cortical walls) has reached an advanced stage.

14. The rate of loss in weight of an Echinocactus, largely due to evaporation, is not correlated with the degree of succulence (proportion of amount of water present to superficial area of body), or with the density of the sap, but is to be attributed to morphological causes.

15. The loss in weight of Opuntia in full sunlight and in diffuse light is

not very different during the first thirty-five days of exposure, and is practically the same after that length of time. The position of the flattened joints in the open may modify the rate of loss.

16. Opuntia desiccating in the open shows an increase in dry weight, but a decrease in hydrolyzable carbohydrate, while the acidity is not markedly different from the normal, though slightly less. Desiccation in diffuse light results in increase of acidity, increase of dry weight (not as pronounced as in the open) and decrease in hydrolyzable carbohydrate.

17. The hydratation capacity of a joint is increased by desiccation in full sunlight, and is decreased by desiccation in diffuse light. The diminished capacity for absorption of water may be due to the high acidity in the latter case.

18. The difference in behavior of the two types of cacti in desiccation and starvation is correlated with definite physical features. Echinocactus has a globoid stem consisting largely of thin-walled cells, in which the accumulated food-material is in the form of soluble carbohydrates. Solid material and accessory colloids are noticeably lacking. The flattened joint of Opuntia is composed of a net-work of fibrovascular tissue. The fundamental tissue is rich in slime or mucilage, and somewhat higher in total hydrolyzable carbohydrates than is the fibrovascular tissue. The loss of water from the large, globose stems of Echinocactus is much more affected by illumination than in the flattened stems of Opuntia. The course of respiration in the thin stems of Opuntia is such that acids formed during the process are present in greater proportion, and vary more widely through the day, than in the large Echinocacti. Some connection with the hydratation of the slimes or mucilages is suggested.

19. Isolated individuals of succulent species survive varying periods when separated from a moist substratum. If the conditions for photosynthesis are inadequate death may ensue from starvation. The disintegration of solid material in diffuse light may be such that the proportion of water in the tissues may be but little changed after several years of depletion.

Loss in weight in full illumination may not greatly exceed 50 per cent of the water present, without producing death by desiccation. The specific action of such excessive loss of water has not been determined.

20. Extended desiccation and starvation made no alteration in the integument of Echinocactus, but in a plant which had been thus treated for seventy-three months the cuticle was thicker than normal while the outer walls of the epidermal cells were thinner. Cytoplasm and nuclei in the epidermal system were reduced, but new cork layers were being formed as in normal plants. Cell division was seen in the epidermal layer at the bottom of the grooves of the stem. The stomata remained permanently open and many were in a collapsed condition. Guard cells of stomata differed from the normal in having the anterior walls thinner as compared with the posterior walls.

21. The palisade layer was thinner in desiccated than in normal plants of Echinocactus. The cytoplasm was reduced to small masses in the angles of the cells, and the nuclei were variously deformed and reduced in size. Vacuoles had disappeared from the nucleoplasm and a thickened granular layer was present in the peripheral portion.

22. The most pronounced effects of desiccation and starvation were exhibited by the cortex of Echinocactus. The changes noted as having been seen in the palisade tissue were followed by the entire disappearance of the protoplasts and the hydrolysis of the cell walls. The consequent disintegration of cell masses formed lacunae as large as 8 cc.

23. Some of the effects of desiccation and starvation were to be found in the medulla of Echinocactus plants undergoing extended desiccation and starvation, but to a lesser degree. Disintegration of cell walls was observed in restricted areas. No change appeared to be produced in the vascular bundles by desiccation and starvation.

24. Early stages of the changes noted above, such as the reduction in cytoplasm and nuclei of cells, deformation and peripheral thickening of nuclei, and hydrolysis of cell walls, were found in plants which had been desiccated in diffuse light for only ten months.

25. An Echinocactus which had been desiccated for forty-two months and then placed under normal conditions in the soil for twenty-two months, did not entirely regain the normal condition. The epidermal system was fairly normal, excepting irregularity in proportional thickness of anterior and posterior walls of stomatal guard cells. Nuclei of the palisade cells were below normal in size, and only one was seen that had regained normal shape. The peripheral, thickened, granular layer was still present in many cases. The cortex also retained irregularities of cell wall and nucleus, as effects of the starvation and desiccation. Recovery was most advanced in the outer part of the cortical region. Cell walls in the outer cortex varied from two to ten micra in thickness while in the inner cortex the variation was from less than one micron to over twenty micra. The inner cortex of this recuperating plant was characterized by some nuclei which were larger than the normal.

GENERAL COURSE OF DEPLETION IN STARVING SUCCULENTS

D. T. MACDOUGAL

The senior author began a series of tests to determine the rate, course and extent of the water-loss in massive succulents in 1908. Selected individuals of Echinocactus, Carnegiea and other plants with a relatively large water-balance growing in the Tucson region were taken from their habitats and placed upon stands which supported them at the height of a meter, in such manner that the light exposure was normal as to angle. Some were put in this position in the open, exposed to the full force of the sun, and were subject to the high midsummer temperatures of the region. Others were placed in laboratory rooms in which the illumination was from ordinary side-windows, and the temperature was rarely altered by artificial heat. Its general course was more equable than that to which the plants in the open were subjected.

Survival in the open generally did not extend beyond two years as the depletion of the water-balance proceeded with such rapidity that more than half of the amount held by the normal plant in a turgid condition would be lost within that time. (See fig. 1.) No plant of any species tested survived a loss of 54 per cent of the original supply.

The course of desiccation under the conditions named has already been described in several papers.[2] Certain features of the variations in weight of the plants under observation, however, remained without adequate explanation. Among these is to be included the fact that the rate of water-loss decreases more rapidly than the ratio of succulence, which is the proportion of water present to the area of the transpiring surfaces. Thus the loss of 8 to 10 per cent of the water originally present in a succulent would be followed by a decrease of 50 per cent or more in the rate of loss. The concentration of the sap resulting from depletion of the water-balance increased the relative amount of ash, but did not increase the amount of acid present, a fact which was soon seen to depend upon the disintegrating action of light. The large, globoid stems of Echinocactus showed an increased proportion of total solids in the sap when desiccation ensued rapidly, as in the full blaze of sunlight, but when the process was allowed to continue into the second year the proportion of total solids decreased to about that of normally turgid plants. (See MacDougal [12], pages 76–78.)

Nearly all of the plants from which the above generalizations were derived were finally destroyed, or perished by starvation or drying. Among the survivors, however, was a large Echinocactus ("No. 7") which had been taken from the soil in November, 1908, and kept in a shaded room for more than six years. Partial records of this plant from 1908 to 1913 have been published. As this plant was taken for detailed examination in connection with the work of the present paper, however, it is necessary to recapitulate its history for the entire period during which it was kept under observation. This history is shown in table I. In continuation of the practice followed in the earlier papers, the weights here presented are those which will serve to show the course of loss during the winter and summer.

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[2] MacDougal, D. T., and Spalding, E. S., The water-balance of succulents. Carnegie Inst. Wash., Pub. 141. 1910. MacDougal, D. T., The water-balance of desert plants. Ann. Bot. **26**: 71–93. 1912. NOTE.—Failure of the senior author to read proof, because of his absence in the Libyan desert, makes necessary the following corrections in this paper: Page 74, 5th line, read "40 grams," instead of "40 kg." Page 76, 2d line, read "4.248 kg." for "42.48 kg." Page, 77, 7th line from bottom, read "32 liters" for "32,000 liters."

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The winter and summer periods in the above table are arbitrarily limited by convenient dates of observation. Weights were obtained at irregular intervals, which might vary in length from a few days to a few weeks. A second arrangement of the data, to show variations during periods roughly corresponding to the years of the calendar, will therefore be useful in presenting other features of desiccation.

TABLE I

Seasonal variations in weight of Echinocactus No. 7

(Arranged to show loss during winter and summer periods)

DATE OF OBSERVATION	WEIGHT	LOSS SINCE LAST WEIGHING	AVERAGE DAILY RATE OF LOSS	REMARKS
	kilograms	*grams*	*grams*	
November 7, 1908	37.595			Taken from soil and placed on support
December 8, 1908	37.040	555	18	Cut surfaces not yet healed
March 23, 1909	36.120	920	9	Winter period
October 1, 1909	34.135	1985	10.3	First summer period
March 22, 1910	33.480	655	3.8	Second winter period.
October 3, 1910	32.055	1425	7.4	Second summer period
March 17, 1911	31.650	405	2.4	Third winter period
October 16, 1911	30.520	1130	5.3	Third summer period
May 16, 1912	30.120	400	1.9	Fourth winter period
September 25, 1912	29.110	1010	7.6	Fourth summer period: rate higher than 1911
March 20, 1913	28.800	310	1.7	Fifth winter period; rate lower than previous year
September 28, 1913	27.825	975	5	Fifth summer period; rate less than 1912
March 6, 1914	27.445	380	2.4	Sixth winter period; rate higher than previous period
October 4, 1914	26.495	950	5.2	Sixth summer period; rate higher than in previous summer
December 7, 1914	26.395	100	1.9	

The greatest rate at which this plant lost weight was 18 g. daily during the first month after its removal from the soil, at which time the freshly cut surfaces of numerous roots and of other abrasions would facilitate loss of water. Chief interest, however, centers in the happenings after the tissues were normally enclosed. Examination of the records reveals the fact that during a short interval in the first summer the maximum rate was 11.6 g. daily. The seasonal average for the same summer was 10.5 g. daily, which fell to 2.9 g. daily. A maximum of 5.5 g. daily, however, occurred in the sixth summer, a rate which was nearly as great as that of the previous summer (fig. 1).

The high maximum rate of water-loss from the plant after more than five years of desiccation may be associated with the fact that a large amount of new tissue was formed at the apex of the globose body during the period April to June, 1914. The rate of decrease in weight of the plant was seen to fall very rapidly, however (fig. 2), and in a manner not explainable by the ratio of water present to the transpiring surface. In an earlier discussion of this matter it was said (MacDougal [12], page 90):

TABLE II

Annual variations in weight of Echinocactus No. 7

PERIOD	NO. OF DAYS	TOTAL LOSS	AVERAGE DAILY RATE OF LOSS
		kilograms	*grams*
November 7, 1908 to October 3, 1909	328	3.443	10.5
October 3, 1909 to October 3, 1910	367	2.080	5.4
October 3, 1910 to October 16, 1911	378	1.535	4
October 16, 1911 to September 25, 1912	345	1.400	4.4
September 25, 1912 to September 28, 1913	368	1.165	3.2
September 28, 1913 to December 7, 1914	435	1.280	2.9
Totals, 6 yrs. 1 mo.	2221	11.043	Average, 5

> Five possible causes, which might have influenced the rate of transpiration of a desiccating succulent, present themselves. These are as follows: 1st, the increased concentration of the cell-sap, which was of such degree in the experiments as to increase osmotic pressures from 4 or 5 to 10 or 12 atmospheres, might retard evaporation from the cell-membranes; 2d, a diminution of the degree of succulence, or proportion of water per unit area of surface present might lessen evaporation; 3d, desiccation may result in alterations in the character of the outer membranes, or of any of the transpiring walls of the plant; 4th, desiccation may stimulate the formation of new tissues or the alteration of existing cells in such manner as to close openings through which water vapour might pass; and 5th, the positions of the surfaces might be shifted in such manner as to vary the exposure and lessen transpiration.

Livingston[3] has recently pointed out that a concentration of the sap even if carried to a point where an osmotic pressure of 100 atmospheres was exhibited, would not give a retardation of more than 10 per cent of the rate afforded by a pure water surface. It is evident, therefore, that this factor is negligible in the present discussion, as the increases in sap concentration that were found were not more than 5 or 6 atmospheres. The rate of loss diminishes so much more rapidly than the estimated degree of succulence in slowly desiccating individuals, that it is impossible to escape the conclusion that other agencies are operative.

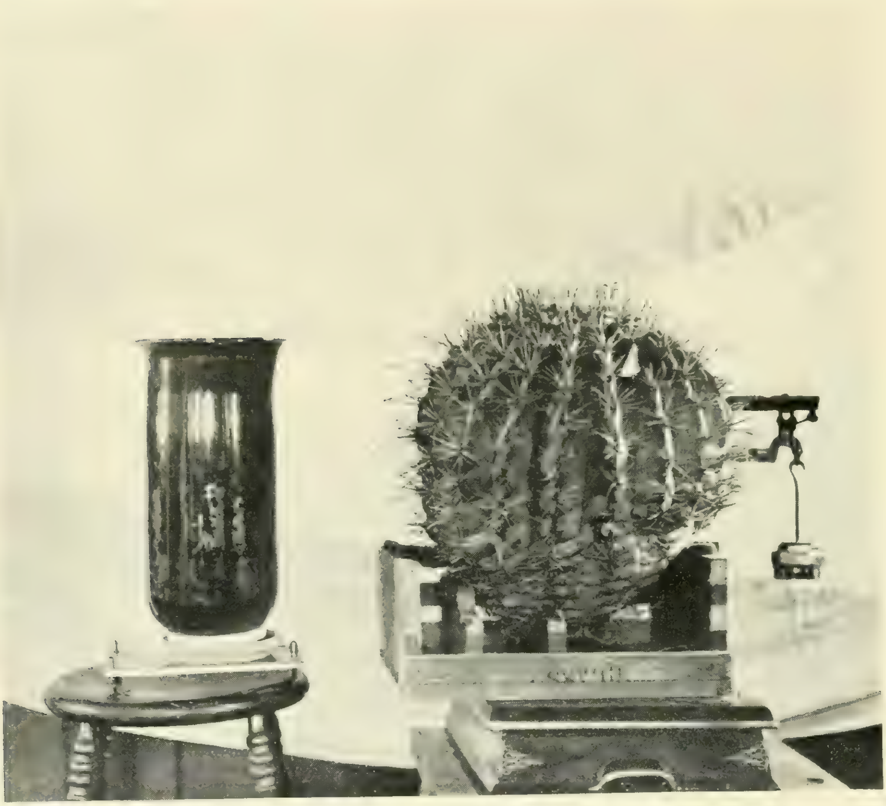

Fig. 1. Echinocactus wislizeni no. 7, December, 1915, after 73 months of desiccation and starvation. The cylinder at the left contains 9.5 kg. of water, the amount lost by the plant during the above period.

No further evidence bearing upon the matter was obtained, and the subject was allowed to rest until opportunity was obtained to make additional measurements. Early in 1914 it was decided to close the career of Echinocactus no. 7 after it had undergone its sixth summer of desiccation. Preparations were made for an anatomical and chemical examination of other individuals which had been subjected to periods of desiccation of varying length. The main inquiries concerned the possible alterations in the tissues and solutions of the cells, as a result of the desiccation, and the fate of the surplus food-material and integral substances of the plasmatic colloids as a result of the continued respiration of the plants which were kept under "starvation" conditions.

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[3] Livingston B. E., The relation of the osmotic pressure of the cell sap in plants to arid habitats. Plant World **14:** 153–164. 1911.

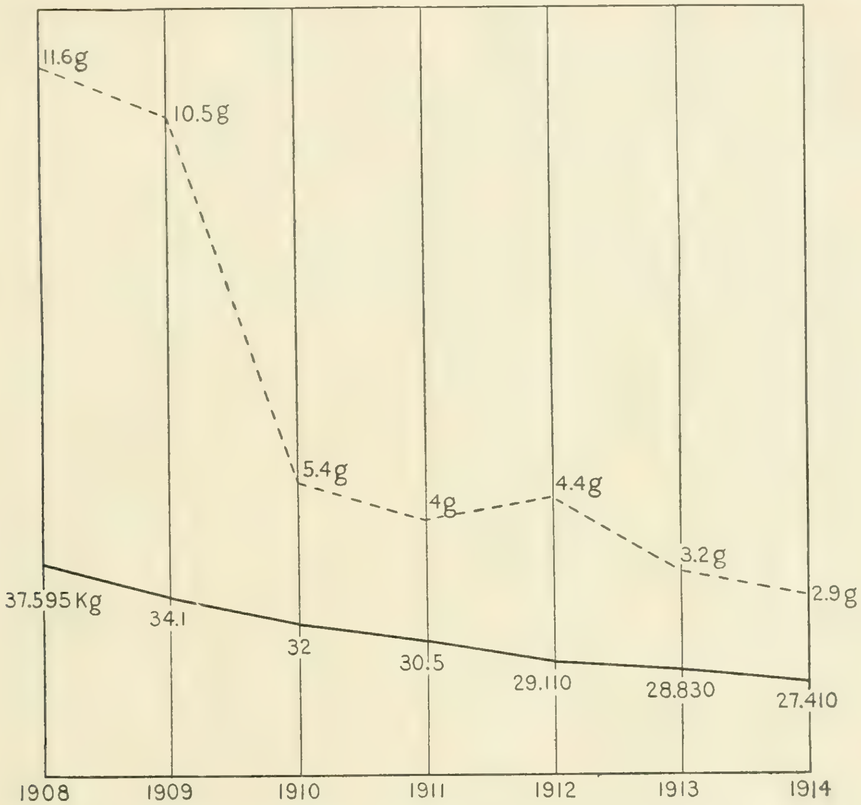

FIG. 2. The solid line shows the course of variation in weight of Echinocactus no. 7, from November, 1908, to December, 1914. The broken line, with its notations, illustrates the variations in rate of loss during this period.

The renewed attention given the matter was chiefly upon the basis of the results achieved by Dr. H. M. Richards and by Dr. H. A. Spoehr in their researches upon the variations in acidity and the general catabolic processes of succulents and other plants. It is to be noted that the starvation and desiccation phenomena discussed are of an extent and duration not previously available to any physiologist. The details are given in the following sections of this paper.

CHEMICAL CHANGES ACCOMPANYING DESICCATION AND PARTIAL STARVATION

E. R. LONG

The attempt to obtain individuals which would illustrate the metabolic changes taking place at intermediate stages of desiccation, was begun in June, 1914, when six healthy plants of Echinocactus were taken up from the slopes west of the Desert Laboratory and placed on supports, three in the laboratory court exposed to full sunlight, and three within the laboratory in diffuse light, in a room with north exposure. The loss in weight was determined by weighing at intervals, as indicated in the following tables, and analyses were made after periods approximately of five, and of eight and one-half months of desiccation under the two sets of conditions. The rate of loss in weight varied little among the plants kept in diffuse light and not a great deal among those exposed to the full sunlight outdoors. Curves of variations of one in the open and one indoors are given in figure 2. The complete record of water-loss for all individuals is given in table III.

TABLE III

Loss in weight of desiccating plants of Echinocactus

DATE	PLANT NO. 21 (FULL SUNLIGHT)		PLANT NO. 22 (FULL SUNLIGHT)		PLANT NO. 23 (FULL SUNLIGHT)		PLANT NO. 24 (DIFFUSE LIGHT)		PLANT NO. 25 (DIFFUSE LIGHT)		PLANT NO. 26 (DIFFUSE LIGHT)	
	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight	Weight	Per cent of original weight
	kg.		*kg.*		*kg.*		*kg.*		*kg.*		*kg.*	
June 24	17.0		14.0		19.2		19.52		26.55		13.00	
July 2	15.7	92.4	13.2	94.3	17.9	93.2	19.24	98.5	26.25	98.9	12.81	98.5
July 9	15.1	88.8	12.6	90.0	17.3	90.1	19.12	97.9	26.02	98.0	12.75	97.7
July 17	14.2	83.5	12.2	87.1	16.5	85.9	19.02	97.4	25.93	97.7	12.68	97.5
Aug. 1	13.3	78.2	11.3	80.7	15.9	82.8	18.82	96.4	25.68	96.7	12.52	96.3
Aug. 22	11.8	69.4	10.1	72.1	14.5	75.5	18.61	95.3	25.39	95.6	12.40	95.4
Sept. 12	11.0	64.7	9.4	67.1	13.6	70.8	18.46	94.6	25.18	94.9	12.25	94.2
Oct. 14	10.1	59.4	9.1	65.0	13.1	68.2	18.24	93.4	24.94	93.9	12.06	92.8
Nov. 2	9.7	57.1	8.8	62.9	12.5	64.6	18.19	93.2	24.90	93.7	12.03	92.5
Nov. 30	9.3	54.7	8.4*	60.0	11.9	61.9	18.10*	92.7	24.79	93.4	11.97	92.1
Feb. 15											11.82*	90.9
Feb. 23	8.3*	48.8										
Mar. 3					10.7**	55.7			24.63*	92.8		

*The plant was then analyzed.

**The plant was then placed in the dark room.

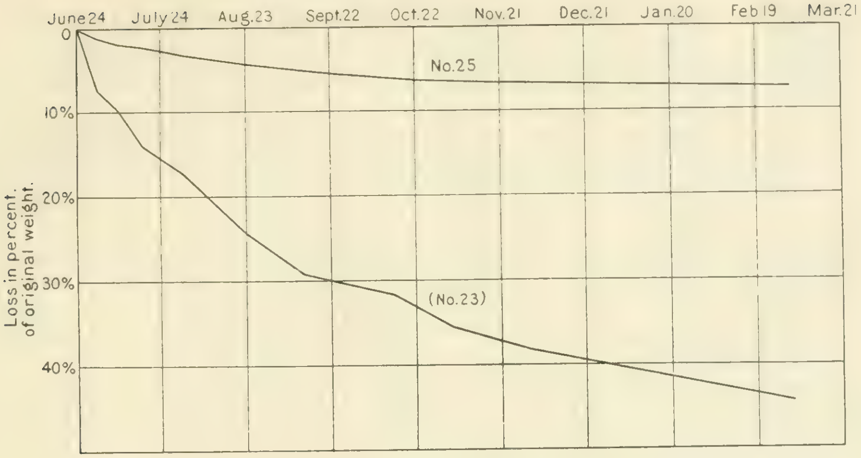

FIG. 3. Tracings illustrating the course of loss in weight of Echinocacti nos. 23 and 25, from June 24, 1914, to March 21, 1915.

The most obvious fact brought out by the data just cited is the relatively great loss of water in the plants exposed in the open, an effect probably due to wind, high temperatures, and other factors tending to increase the evaporative action of the air. The gradual flattening of the curves, indicative of a drop in the rate of water-loss, is also at once appparent. The immediate explanation for this phenomenon must be sought on morphological grounds. The mere increase in concentration of the cell sap would not be sufficient to cause the observed drop in the rate of water loss, although it may be a factor in causing morphological changes. It is a well known fact that within rather wide limits water evaporates from the moist surfaces of colloidal solutions of varying concentration at approximately the same rate as from the surface of distilled water. It is perhaps not out of place to cite an experiment to illustrate this, in which 50 cc. of distilled water and the same amount of a 2 per cent gel of gelatine were used, in similar vessels.

A slightly larger loss from the water surface is noted after eight days at a time when the concentration of the gel has increased more than 20 per cent.

TABLE IV

Evaporation from water surface and from surface of gelatine gel

DATES	TOTAL LOSS FROM GELATINE GEL	TOTAL LOSS FROM PURE WATER
	grams	*grams*
January 8, 2 p.m.	0	0
January 9, 10 a.m.	1.26	1.16
January 13, 2 p.m.	6.92	6.97
January 16, 2 p.m.	11.28	11.44

However, it should be noted that the graph for the rate of loss in weight of the gel is practically a *straight line*, and not a flattening curve, as is the case in the desiccation experiments just recorded. Such results make it apparent that the decreasing rate of loss in weight can not be satisfactorily attributed to increased sap concentration directly, although it is within the range of probability that heightened concentration of the cell-solutions might exert a direct effect upon the membranes, which might, in turn, result in lessened water-loss.

The chemical analyses were confined to those points where changes might be expected as a result of prolonged desiccation and catabolism without repair. As a routine procedure, determinations were made of the dry weight, of the density and total solids of the sap, of the acidity, and of the carbohydrate material available for nutriment. In some cases the protein and fat content and the hydratation or swelling power, were also determined.

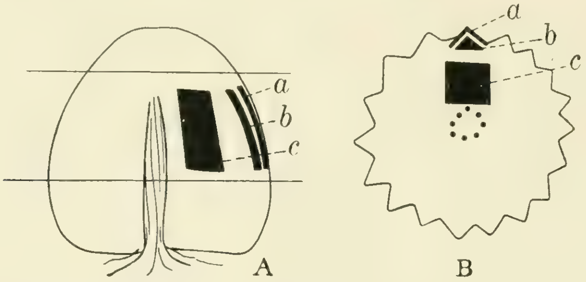

Fig. 4. Diagrams of cross (B) and longitudinal (A) sections of stem of Echinocactus, to indicate parts of the cortex from which samples for chemical and microscopical examination were taken.

In all cases the general plan was followed, of analyzing material from three arbitrarily defined tracts in the plant, which were localized as indicated in figure 4. These regions are designated as *a*, *b*, and *c*, *a* and *b* being in the external cortex so near the general surface that they might be included in the spine-bearing ridges characteristic of this plant, while *c* was taken from a position within a few centimeters of the central cylinder, The last sample (*c*) probably represents nine-tenths of the total mass of the cortex.

The methods employed were all standard ones, easily applied. The dry weight was determined at 100°–105°C, on representative samples of the ground and uniformly mixed material from each of the three tracts. The sap was expressed and strained by means of a fruit press, and its density measured with a Westphal balance. The total solids of the sap were found by evaporation on the water-bath and drying at 100°–105° in the oven. Fehling's solution was used for the sugar determinations. The soluble sugars were estimated after alkalination of the expressed sap and filtration from precipitated hydrates and carbonates. Reducing sugars were determined directly, by titrating the alkalinized sap directly with Fehling's solution, the copper ferrocyanide test plate method being used for accuracy in the end-point. "Soluble non-reducing sugars" were estimated after one hour's hydrolysis of the alkalinized sap on the water-bath, the material being made 10 per cent acid with HCl. The resulting solution was neutralized, diluted to a standard, and titrated with Fehling's solution, the value for reducing sugars being subtracted from the figure found, to give the correct figure for soluble *non*-reducing sugars. It was noted that the sugar content of the alkalinized and unalkalinized sap after hydrolysis, was the same; that is, that no hydrolyzable carbohydrates were thrown down in the precipitating process.

"Total hydrolyzable carbohydrates" (taken to include all carbohydrates existing in hydrolyzed form) were determined by subjecting 50 g. samples of tissue to four hours hydrolysis with 5 per cent hydrochloric acid, a vigorous ebullition being maintained under the reflux condenser, neutralizing, diluting to 1000 cc., and titrating against Fehling's solution. It is taken for granted that this strength of acid does not appreciably affect cellulose, and that the value obtained roughly measures the carbohydrate which may undergo catabolism in the organism. It was assumed when the experiments were begun that the sugars were chiefly of the hexose type and the analyses described above were carried out to determine reducing sugars, sugars on inversion, and insoluble polysaccharides, it being supposed that the sugars thus determined were dextrose and fructose, sucrose, and starch. However, toward the close of the experiments, analyses made by and at the suggestion of Dr. J. H. Long, who was visiting the Desert Laboratory at the time, brought out the fact that a considerable proportion of the sugars were of the pentose type. Accordingly under "reducing sugars" pentoses as well as dextrose and fructose are probably included, and under "soluble non-reducing sugars" soluble pentosans (as constituents of gums) as well as disaccharides, while the term "total hydrolyzable carbohydrate" probably covers insoluble pentosans and hemicelluloses, as well as starch. While this complicates the chemistry of our problem considerably, the actual interpretation of results from a physiological point of view, is not so much modified as might at first be thought, since we are concerned more with amounts than with kind, bearing in mind, however, the fact that pentoses are typically more stable than hexoses, and perhaps less readily broken down in catabolic processes. Pentoses were estimated in the few cases examined by the Jolles method, by which furfuraldehyde is distilled from the hydrolyzed material and treated with an excess of sodium bisulphite, the amount of excess being determined by titrating back with iodine.[4]

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[4] See: Abderhalden, Handbuch der Biochemischen Arbeitsmethoden. **2**: 135. 1910.

Acidities are expressed in terms of cubic centimeters of tenth-normal NaOH required to neutralize 1 cc. of the expressed sap (to phenol phthalein). Crude protein was estimated by simple total nitrogen determination, and crude fat by ether extraction. Hydratation, or water-absorption, is expressed in terms of the percentage of the original weight taken up by pieces of tissue of approximately the same weight and surface area, in two hours time in distilled water at equable room temperatures.

Analyses were made of plants of Echinocactus as follows: Nos. 7, 24, 25 and 26, desiccated in diffuse light within the laboratory; nos. 21 and 22, desiccated in full sunlight within the laboratory court; and nos. 34, 35 and 36, normal—i.e., just taken up from their natural habitat. The results of the analyses are summarized in table V. Carbohydrate, protein and fat values are expressed as percentage on the basis of dry weight. The figures given for the sugars are all calculated on the assumption that 10 cc. of Fehling's solution is reduced by 0.05 g. dextrose or pentose. In view of a lack of knowledge, in most cases, of the proportions of pentosan, hemicellulose, and starch separately, corrections for the excluded molecule of water in the dehydrated carbohydrates $(C_6H_{10}O_5)_x$ and $(C_5H_8O_4)_x$ are not made under the heading "Total hydrolyzable carbohydrates;" the figures given in this column represent the total carbohydrate in the hydrated form after hydrolysis, i.e., as $C_6H_{12}O_6$ and $C_5H_{10}O_5$. This fact will be taken into account below, in the consideration of calculations made on the basis of dry weight.

The most interesting feature brought out by table V is the fact that after six years of desiccation, and after a lost of 29.3 per cent of its weight chiefly by water evaporation, Echinocactus no. 7 still averaged about 94 per cent of water, or practically the same as the average of the three normals nos. 35, 36 and 37. The explanation follows easily enough and will be brought out in the consideration of the sugar metabolism immediately following. But at the time of analysis the result was thought startling enough to warrant relocating the basal third of this plant, which had been cut off as usual and discarded, had been lying in the open with the cut surface exposed, for nine days, and had undoubtedly lost a considerable amount of water. A representative sample of this basal portion averaged 91.1 per cent of water. Evidently no serious error had been made in the dry weight determinations, which had been made in duplicate. We were then convinced that changes in the composition of the dry weight, indicative of a loss parallel to the water depletion were to be expected.

The dry weight of samples and the density and total solids of the sap are so closely related with sugar concentrations that they will not be considered separately. It is in the sugars and sugar producing constituents that we find the most marked and most interesting changes, changes which, in turn, explain the variations observed in the dry weight, density and total solids. As might be expected these alterations are at their maximum in the plant starved and desiccated the longest, no. 7.

In no. 7 we find a greatly reduced content of total hydrolyzable carbohydrates, the latter after hydrolysis forming only 11.1 per cent of the dry weigh of the cortex of the plant (sample *c*). The average content of the normal plants studied, in hydrolyzable carbohydrates, in the same samples (*c*), which, as has been remarked, represent about nine-tenths of plant, is 26.1 per cent of the dry weight, and one of the plants (no. 35) is probably exceptionally low in this respect.

Something like 15 per cent of the dry weight of the cortex (a little less than 15 per cent as this calculation is in terms of hexoses and pentoses, while a large proportion of the total carbohydrate actually existed as the dehydrated polysaccharides) had disappeared in the course of the long period of low illumination and consequent starvation. In the peripheral tenth of the plant the decrease in sugar content below the normal was not so great. The average content of total carbohydrate in regions *a* and *b* for nos. 34, 35 and 36 was 32.5 per cent of the dry weight, and in the same region in no. 7, 23.3 per cent. Thus, if at the start of its period of starvation, no. 7 had the sugar content in the outer region of a plant with the average sugar concentration found in nos. 34, 35 and 36, it had lost in the course of six years, 9.2 per cent. The average loss for the entire plant, by the same method of calculation, would be about 13 or 14 per cent. Unfortunately we have no way of knowing what the sugar content of no. 7 actually was when it was brought in for experimental purposes. It is to be noted that nos. 34, 35 and 36 were analyzed in early January, when the winter was well under way, while no. 7 came out of the ground on November 7, at the close of the summer—that is, at the close of the period when the conditions for sugar manufacture were at their best, but when disintegration was also very high.

Whatever is true, no. 7 had consumed more than 13 or 14 per cent of its dry weight. It had lost 29.3 per cent of its original weight and still maintained its normal, high proportion of water. A certain proportion of this 29. per cent must have been lost in the combustion of solid material itself, but by far the greater part disappeared in water evaporation. To lose some 29 per cent of its weight by water evaporation and maintain its original proportion of water, it must have lost an equivalent proportion of its dry weight. The loss must have occurred almost entirely in the carbohydrate portion. No loss in inorganic constituents was possible and no loss in protein or lipoidal substances actually took place. The reduction in acidity, to be described later, accounts for a small amount of the loss. The greatly lowered total hydrolyzable carbohydrate content accounts for a very large amount of it, but unless no. 7 was very much richer in this portion of its composition than the normal plants studied in connection with it, not for all. Could other carbohydrates, for example, cellulose and the other substances forming the fibrous system of the plant and ordinarily considered very stable, have taken part? Apparently they did. As will be pointed out in the discussion of morphological changes, large lacunae were found on section of the plant, scattered through the cortex, where tissue had disappeared completely. A cytolysis involving some of the most stable of the organic substances of the plant's tissues had taken place, leaving the products to follow the fate of the other carbohydrates. It is upon this cytolysis that we must fall back, to explain the balance of dry weight loss which must have paralleled the loss by water evaporation.

So far we have been unable to reproduce this cytolysis experimentally in the laboratory. It is evidently a process requiring a very long time.

As was noted above the drop in total sugar content was not so great in the chlorophyllose tissue as in the deeper parts. No. 7 was not in darkness. A certain amount of photosynthesis was undoubtedly going on enough to furnish the outer parts with something under their normal supply of sugar, but leaving no margin for translocation to the interior.

The drop in the sugar content of no. 7 was very noticeable in the concentration of sugars dissolved in the cell sap. The reducing sugars were very much lower in amount in no. 7 than in the normals, nos. 34, 35 and 36. The soluble non-reducing sugars were also much lower in amount than in nos. 24, 26 and 25, which were "starved" for a much shorter period. This low concentration of soluble sugars, as compared with a normal plant, explains the paradoxical fact that after six years of concentration by desiccation, the sap of no. 7 has actually been lowered in density (see table V), being the lowest recorded in the entire series.

As intermediates between the normals, nos. 34, 35 and 36, on the one hand, and no. 7, on the other, nos. 24, 26 and 25 are not entirely satisfactory. Time had been allowed for a loss in weight not yet amounting to a third of that which had taken place in no. 7, and no appreciable sugar destruction had yet taken place. In fact, the figures would indicate (total hydrolyzable carbohydrate, table V) that a slight concentration was taking place. The change is perhaps not unaccountable on the ground of individual variation.

Comparing nos. 24, 26 and 25 with the normals, nos. 34, 35 and 36, however, we are struck by two other points. The first of these is in the distribution of the reducing sugars. Normally these are found in greatest amount in the outer tissues, where they are being formed, and in progressively decreasing concentration as the center is approached. In nos. 24, 26 and 25 the reverse condition is observed. The concentration is least in the exterior and progressively increases as the center is approached. This is probably to be referred to the slow rate of photosynthesis when the plants were brought indoors. Such an effect would naturally soon be realized if the normal movement of food material toward the interior kept up for any length of time after the rate of food production in the external layers was so greatly lowered. In connection with the change in distribution of reducing sugars, a drop from the normal in their total amount is also noticed. The second point of departure from the normal observed in nos, 24, 26 and 25 is in the soluble non-reducing sugars. The latter apparently exist in low concentration, or, at times, not at all, in the sap of normal plants. They are present in considerable concentration in the sap of all of the desiccating plants. The fact that they are present in really large amount in the sap of no. 22, a plant desiccated in full sunlight and obviously laying up sugars, and in much less amount in no. 21, a similar specimen, which was rapidly breaking up, however, suggests that they are products of anabolism rather than catabolism.

Nos. 22, 21 and 23 were individuals which were desiccated in full sunlight, that is, under such conditions that they could manufacture sugars. Of these, nos. 22 and 21 were analyzed after periods of desiccation of five and eight months respectively, and no. 23 was put away in a dry dark-room to be studied at a later date for the effect of a lowered rate of water evaporation accompanying a destruction without formation of sugars. The most noticeable features in the analytical results for no. 22, were the high density of the sap and its high content of non-reducing sugars, just touched upon above. The latter, combined with the concentration of dissolved salts occurring, accounts for the former. The laying up of soluble non-reducing sugars was sufficient to cause a marked rise in the proportion of the dry weight present as hydrolyzable carbohydrate. The dry weight had been raised by the desiccation in proportion to the water lost. No. 22 was to all appearances in healthy condition in all respects except water content, and would probably have survived had it been returned to the soil.

No. 21, on the other hand, had possibly suffered to a point where it could not be revived. It was in a state of low turgor, and the tissues of the side most exposed had collapsed. Everything indicates that it was breaking up rapidly. In spite of a water-loss exceeding that of no. 22 by 11.2 per cent, the density of the sap of the cortex of no. 21 was the lower of the two. The total solids of the sap were considerably lower than might have been expected. Determinations of total solids were not made for no. 22, but figures for a comparable specimen are available. Echinocactus no. 10 of a former series (MacDougal [12]) had been similarly exposed, and at the time of analysis had practically the same cortical sap density as no. 22 (1.035), and a total solid content of 7.1 per cent. It seems at first rather surprising that no. 21, which had lost more water than either no. 10 or no. 22 should have a lower sap density and a lower content of dissolved material. The explanation lies in the greatly reduced sugar content of the sap of no. 21. Comparison of the protocols for nos. 22 and 21 shows that both the reducing and soluble non-reducing sugars exist in much lower concentration in the sap of the latter. The total hydrolyzable carbohydrate is also much lower in no. 21. Just why this condition should occur in a plant afforded full opportunities for the manufacture of sugars, at least as regards illumination, is not easy to say. The chlorophyllose tissue still bore a healthy appearance to the unaided eye. However, the carbohydrate content, so low compared with that of its fellow no. 22, and the higher acidity, would seem to indicate that a rapid combustion was taking place, with nothing like adequate repair. This consideration probably explains the phenomenon noted by MacDougal [12] in a similar case. Echinocactus no. 10 dried out in the open for seven months, losing 33.3 per cent of its weight, and at the time of analysis had a content of dissolved solids of 7.1 per cent. No. 13 of the same series dried out in the open for thirteen months, lost 48.3 per cent of its weight, and at the time of analysis had *a total dissolved solid content of only 3.7 per cent.* Sugar determinations were not made. Had they been made it is likely that a greatly reduced sugar content would have been found in the sap of no. 13. Like no. 21, no. 13 was probably rapidly breaking up. Both plants were, in all likelihood, in advanced stages of starvation.

In connection with carbohydrate metabolism, we should consider some of the intermediate products of the destruction; viz., the organic acids. In the light of recent work, we do not expect to find an increase in acidity necessarily accompanying a concentration of the sap. The effect of light and warmth in destroying the acids accumulating in the sap of succulents from catabolizing sugars, has been measured by Richards,[5] and the course of destruction has been followed in detail by Spoehr.[6] In the course of the experiments recorded here very high acidities were never encountered. The range of acidity of the sap of Echinocacti at the season when nos. 21–26 were taken up from the ground (summer, 1914) was followed carefully,[7] and the average at the time of day when nos. 21–26 were analyzed later (8–10 a.m.) was found to be as follows: *a*, 0.297; *b*, 0.272; *c*, 0.196.

The specimens from the same tracts in Echinocactus no. 24, analyzed in the late fall, are below this average in acidity, and this in spite of the fact that for five months no. 24 had not been getting as much illumination as previous to the commencement of its desiccation—a condition favorable to rise in acidity. However a considerable variation is manifested in normal plants, and it is possible, too, that the explanation lies in the event of a generally lowered metabolism in consequence of the cutting off of the water supply. In nos. 26 and 25, however, which were analyzed some months later, a progressive rise in acidity is noticed. It is altogether probable that this is due to the prolonged low illumination and colder temperatures prevailing. Between September 1 and December 1, temperatures of the air surrounding the plants varied between 95° and 65° F., and between 65° and 40° F. between the latter date and March 1. Combined with the lower temperatures of the latter period was the increased amount of darkness, which in the winter of 1914–15, a very rainy one, was unusually great. Thus, the destructive influences of illumination and warmth being removed to a greater extent than previously, conditions favorable to a rise in acidity are at hand, and we are not surprised to see this rise actually taking place as noted in Echinocacti nos. 26 and 25. (See table V.)

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[5] Richards, H. M., Respiration and acidity in plants. Carnegie Inst. Wash. Pub., Washington, 1915. (*In press.*)

[6] Spoehr., H. A., Photochemische Vorgänge bei der diurnalen Entsäurung der Succulenten. Biochem. Zeitschr. 57: 94–111. 1913.

[7] Long. E. R., Acid accumulation and destruction in large succulents. Plant World, 1915. (*In press.*)

The lowest acidities recorded anywhere in the entire series, were in Echinocactus no. 7, desiccated in diffuse light more than six years. It is not difficult to explain this. As emphasized above, the sugar content of this specimen had reached a very low state, and as acidity under such conditions is apt to be roughly parallel to the total sugar—a fact illustrated in the protocols for nos. 7, 24, 26 and 25—we should expect an accompanying reduction in acidity, provided a sufficient length of time was allowed for the agencies destructive to acidity to bring about a state of equilibrium. No. 7 had probably long since passed through the stage of acidity concentration recorded in nos. 26 and 25, and at the time of analysis was evidently in a final stage of minimum metabolism.

It would be hazardous to attempt to draw conclusions from the acidity values of the other cacti represented in the tables. These were all exposed to full sunlight and were undoubtedly undergoing great diurnal variations in acidity. In the routine procedure of analysis the acidity of these plants was determined about 11 a.m., after some hours of sunlight, and accordingly comparisons cannot fairly be made with the specimens kept indoors under more equable conditions of light and temperature.

It would not be unnatural to suppose that the tissues of a plant which had steadily dried out for more than six years, would have an abnormally high power of absorbing water. Certainly the osmotic pressure of the cells would be raised by the concentration of dissolved salts. However there is another factor to be taken into account, which has been receiving a steadily increasing prominence in the literature of recent years. This is the feature of colloid hydratation.[8]

It has been found that the tissues of platyopuntias take up water readily from neutral and alkaline media, and somewhat less readily from an acid surrounding. Echinocactus has not been investigated, but it is likely that conditions are similar. In Echinocactus no. 7 we have a balanced state of affairs, an increased power of absorption, due to a higher osmotic pressure resulting from salt concentration, and a greatly lowered carbohydrate content (see table V) making for a weaker absorbing power, together with a low acidity, which may or may not be favorable to hydratation. Echinocactus no. 25, which had dried out relatively little (7.2 per cent loss in weight as

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[8] Long, E. R., Growth and colloidal hydratation in cacti. Bot. Gaz. **59**: 491–497. 1915.

TABLE V

Results of analyses of tissues of Echinocactus

PLANT NO. AND TREATMENT	TOTAL LOSS, PER CENT OF ORIGINAL WEIGHT	TISSUE SAMPLE	DRY WEIGHT, PER CENT OF TOTAL WEIGHT	SAP DENSITY (WATER = 1.00)	TOTAL SOLIDS IN SAP, PER CENT OF TOTAL SAP WEIGHT	SAP ACIDITY, $\frac{N}{10}$	TOTAL HYDROLYZABLE CARBOHYDRATE, PER CENT OF TOTAL SOLIDS	TOTAL REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	TOTAL NON-REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	HYDRATATION (WATER ABSORBED), PER CENT OF ORIGINAL WEIGHT OF SAMPLE
No. 7 Desiccated in diffuse light 6 yrs. 1 mo. Analysis begun Dec. 8, 1914.	29.3	*a*	9.50	1.010	......	0.144	22.3	0.09	0.11	9.4
		b	7.96	1.008	......	0.104	24.2	0.06	0.10	11.6
		c	5.75	1.013	......	0.148	11.1	0.04	0.06	11.2
No. 24 Desiccated in diffuse light 5 mo. 9 da. Analysis begun Dec. 3, 1914.	7.3	*a*	10.9	1.018	......	0.212	31.1	0.16	0.30	......
		b	9.3	1.018	......	0.192	28.6	0.21	0.25	......
		c	8.4	1.020	......	0.156	23.8	0.31	0.46	......
No. 26 Desiccated in diffuse light 7 mo. 23 da. Analysis begun Feb. 15, 1915.	9.1	*a*	9.6	1.020	4.05	0.220	32.9	0.10	0.80	......
		b	7.7	1.020	3.84	0.212	27.6	0.18	0.65	......
		c	7.5	1.020	3.87	0.204	25.6	0.50	0.50	......
No. 25 Desiccated in diffuse light 8 mo. 6 da. Analysis begun Mar. 2, 1915.	7.2	*a*	10.2	1.019	3.51	0.588	33.8	0.05	0.62	9.3
		b	7.5	1.017	3.12	0.460	33.3	0.14	0.46	13.2
		c	6.0	1.016	3.04	0.212	28.8	0.33	0.31	10.9
No. 22 Desiccated in full sunlight 5 mo. 6 da. Analysis begun Dec. 1, 1914.	40.0	*a*	14.3	1.016	......	0.244	44.3	0.15	1.28	......
		b	13.3	1.027	......	0.208	44.2	0.13	1.48	......
		c	11.3	1.034	......	0.156	43.4	0.10	2.67	......
No. 34 Not desiccated. Removed from ground 2 da. before analysis, which was begun Jan. 11, 1915.	......	*a*	5.84	1.013	......	0.172	32.3	0.53	0.14	......
		b	4.20	1.011	......	0.156	35.7	0.42	0.03	......
		c	3.55	1.011	......	0.128	29.6	0.10	0.05	......

TABLE V—Continued

PLANT NO. AND TREATMENT	TOTAL LOSS, PER CENT OF ORIGINAL WEIGHT	TISSUE SAMPLE	DRY WEIGHT, PER CENT OF TOTAL WEIGHT	SAP DENSITY (WATER = 1.00)	TOTAL SOLIDS IN SAP, PER CENT OF TOTAL SAP WEIGHT	SAP ACIDITY, $\frac{N}{10}$	TOTAL HYDROLYZABLE CARBOHYDRATE, PER CENT OF TOTAL SOLIDS	TOTAL REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	TOTAL NON-REDUCING SUGARS IN SAP, PER CENT OF TOTAL SAP WEIGHT	HYDRATATION (WATER ABSORBED), PER CENT OF ORIGINAL WEIGHT OF SAMPLE
No. 35 Not desiccated, removed from ground 9 da. before analysis which was begun Jan. 18, 1915.		*a*	8.12	1.018	......	0.204	28.6	1.11	0.08	......
	......	*b*	6.23	1.018	......	0.204	29.1	0.91	0.05	......
		c	5.42	1.018	......	0.220	19.7	0.39	trace	......
No. 36 Not desiccated, removed from ground 12 da. before analysis, which was begun Jan. 21, 1915.		*a*	10.18	1.018	......	0.172	35.0	1.13	0.03	......
		b	8.77	1.017	......	0.140	34.5	1.28	0.0	......
	......	*c*	8.66	1.016	......	0.136	29.1	1.02	0.0	......
No. 21 Desiccated in full sunlight 8 mo. Analysis begun Feb. 23, 1915.		*a*	17.8	1.028	5.48	0.400	25.3	0.09	0.58	......
	51.2	*b*	15.7	1.028	5.40	0.260	30.1	0.03	0.56	......
		c	12.9	1.028	5.49	0.192	25.8	trace	0.37	19.3
No. 23 Desiccated in full sunlight 8 mo. 10 da. placed in dark room Mar. 6, 1915.	44.3									

against 29.3 per cent in no. 7) and had a high content of hydrolyzable carbohydrates—for the most part probably substances of colloidal nature—was examined as to its swelling power for comparison. Not a great deal of difference was found (see table V). The swelling of no. 7 is slightly greater in the case of *a* and *c* (fig. 4), and appreciably less in the case of *b* (fig. 4). It seems likely that, as compared with no. 25, the tendency to increased absorption in no. 7, due to a higher osmotic pressure, was practically counterbalanced by the much lower colloidal content and accordingly sections from the two plants swelled to about the same degree. In Echinocactus 21, which had dried out in the sunlight to such an extent that it had lost half its water,

and presumably had a high absorbing power on both increased osmotic pressure and high colloid content, the imbibition of water by the deep cortex (*c*) under similar conditions of time and temperature, was 19.3 per cent or nearly twice that of nos. 7 and 25.

Nitrogenous and lipoidal constituents form so small a proportion of the tissue of the Echinocactus that changes in their amount are of relatively little importance in the gross effects of catabolism, and for this reason they were not taken into account extensively in our conduction of the problem, which was concerned with the greater, more readily visible changes. That alterations, however, in their amount or character, as a result of the rigorous conditions to which the plants may be exposed, would have great effect upon the course of the rest of the metabolism, is altogether likely, for in all probability the substances concerned are essential to the life of the plant in nuclear metabolism and may play a part in the permeability of the membranes. On the other hand, these substances would be the last constituents to break down and disappear under conditions of starvation without adequate repair. Therefore it is not surprising that no distinct changes were noted on subjecting a desiccated and a normal specimen to protein and lipoid analysis. The slight differences found may be assumed to lie within the ordinary limits of variation existing among normal plants. The data of protein and fat content, as obtained from these analyses, are presented in table VI.

TABLE VI

Contents of crude protein and of crude fat in tissues of desiccated and normal Echinocactus

ECHINOCACTUS	TISSUE SAMPLE	CRUDE PROTEIN	CRUDE FAT
		per cent of total dry weight	*per cent* of total dry weight
No. 7, desiccated 6 yrs. 1 mo.	*a*	2.50	0.75
	b	2.63	0.80
	c	0.81	0.97
No. 35, Normal	*a*	2.31	1.11
	b	2.31	0.93
	c	0.50*	1.01

*Approximate.

It should be noted that the value of the protein determinations here given must be discounted somewhat by the fact that a certain amount of nitrate (which would be the end product of protein catabolism) would be included in the "Total N" determination. The greater part would probably go off as nitric acid in the course of a Kjeldahl analysis.

The protein of *c*, Echinocactus no. 35, was not determined with absolute accuracy because of an accident in the titration.

From the chemical data recorded above we can reconstruct with some accuracy of detail the story of starvation in a desiccating succulent. When one of these massive plants is deprived of its water supply and brought into diffuse light where its photosynthetic activity is greatly lowered, the first effect is a simple loss of water, the rate of which, however, gradually falls. The first change noticeable in the food material is in the concentration and distribution of the soluble sugars. The rate of production of reducing sugars has notably decreased, and those present in the sap at the initiation of the starving period are removed by translocation and catabolism. A distribution of these sugars which is the reverse of the normal occurs; the concentration becomes greatest in the interior and progressively less as the surface is approached. At the same time soluble non-reducing sugars, ordinarily present to slight extent only in the sap of normal plants, appear in appreciable concentration, in a manner not easily explainable. As the period of low illumination is continued, the acidity begins to rise.

Sometime after the first year the content of carbohydrate available for nutriment begins to drop. The concentration of dissolved sugars is probably the first to be lowered, and is followed by a breaking up of the insoluble polysaccharides at a rate exceeding their restoration. A resultant fall in the dry weight roughly parallel to the water loss is thus established, of such effect that the plant may maintain its normal high proportion of water or even exceed it. Photosynthesis continues to a certain extent in the outer cortex, in which the sugar content does not vary greatly from the normal. But the drain is felt in full force in the interior in the great body of the plant. The original distribution of reducing sugars, in which the latter steadily decrease in amount as the center is approached, is restored. Only their concentration is greatly reduced. At the same time the amount of soluble non-reducing sugars is lowered. Coincident with this lowering of sugar concentration, in spite of continued water evaporation, the sap density drops to a point even below that of a normal plant.

As the amount of carbohydrate capable of undergoing catabolism decreases, the acidity, after its preliminary rise in response to the removal of light, begins to fall, not because its rate of destruction is increased, but because its production through the catabolism of sugar is so greatly lowered. A point is reached where even in diffuse light, acid destruction proceeds more rapidly than acid formation. Eventually cytolysis begins. During all this time the protein and fat content remains normal.

After six years of desiccation and a loss of a quarter of its weight, we may have a plant with a proportionate content of water the same as that of a normal plant, in the neighborhood of 94 per cent, and with a sap density not differing greatly from the normal. But a chemical analysis shows that the water ratio has been maintained only because the carbohydrate food supply has burnt at a rate parallel to the water-loss, and has become greatly lessened.

Consequently the tissues have no increased power of hydratation, for the colloid substances active in the absorption of water by normal tissues, have been to a large extent removed.

Desiccation of Platyopuntias

The principal features of desiccation of the flattened joints of the opuntias of this region being known to be something different from those presented by the globose Echinocacti, it was deemed essential to follow a number of these plants through the earlier stages of depleted water-balance in order to compare the variations in weight and water-content with those discussed in the previous section. The material consisted of twenty-four turgid joints of *Opuntia discata* taken from two plants growing near the chemical building of the Desert Laboratory on September 28, 1914. The separate joints were taken from terminal portions of the plant and were cut cleanly at the base in such manner as to be as nearly equivalent as possible in evaporative capacity. Six were arranged on a wooden support in an upright position in the open, exposed to the full illumination, the planes of the joints being north and south; six were placed near these but in a horizontal position, being turned once every week so that the two sides were alternately uppermost; six were similarly placed in an upright position in the diffuse light in the middle of the largest room in the main building of the Desert Laboratory, and six in a horizontal position in the same place. Weights were taken as given in table VII, and as graphically illustrated in figure 5.

TABLE VII

Water loss of desiccating opuntias

DATE	6 PLANTS OUTDOORS, UPRIGHT	6 PLANTS OUTDOORS, FLAT	6 PLANTS INDOORS, UPRIGHT	6 PLANTS INDOORS, HORIZONTAL
	per cent of original weight	*per cent* of original weight	*per cent* of original weight	*per cent* of original weight
September 30	100.0	100.0	100.0	100.0
October 2	97.1	97.7	98.2	97.
October 5	96.1	96.7	97.1	96.7
October 9	93.4	95.1	95.9	95.6
October 14	91.1	93.2	94.7	94.0
October 19	88.6	91.9	93.3	92.8
October 26	86.2	90.2	92.2	91.7
November 2	85.0	89.2	91.3	90.7
November 16	83.0	87.7	89.8	89.6
November 30	82.0	86.8	88.7	88.5
December 30	80.7	85.7	87.5	87.5

The most noticeable feature brought out by comparison with the data obtained from the Echinocacti is that the difference in the rate of decrease in weight between plants in the diffuse light and those in the open at much higher temperatures and exposed to full sunlight is comparatively small at all times, being practically identical after the first thirty-five days of exposure. At this time Echinocacti were losing weight at a rate four times as great in the open as in the shaded room.

It is to be noted that platyopuntias drying out indoors lose water more rapidly than Echinocacti under the same conditions, while platypuntias desiccating in the open in full sunlight lose water considerably less rapidly than Echinocacti drying in the open. The latter fact may be of some ecological significance in explaining the vastly wider distribution of the platyopuntias.

The effect of *position* upon water loss, is well shown in table VII. Platyopuntias desiccating in the upright position, exposed in the open, lose water more rapidly than similar joints lying horizontal. This is probably an effect of the different amount of illumination operating in each case. The plants set upright were exposed to sunlight and sky radiation on both sides throughout the day, while in the plants lying flat one side was naturally shaded. It should be noted that the frames were so constructed that the joints were as much exposed to the air on one side as upon the other. As might be expected this effect of position does not appear in the results for the plants desiccating indoors, where the factor of illumination is so greatly modified.

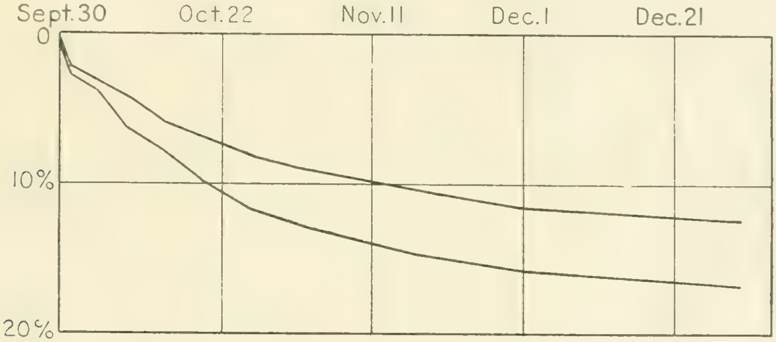

FIG. 5. Tracings illustrating course of loss in weight of a platyopuntia, in terms of original weight. The upper line expresss average of 12 joints desiccating in diffuse light of shaded room, the lower line expresses the average of 12 joints desiccating in the open near the Desert Laboratory.

Chemical analyses undertaken after three months of desiccation were confined to acidity and total hydrolyzable carbohydrate determinations. The latter were made in the same manner as for the Echinocacti.

The presence of slimes renders the expressing of the sap for determination of acidity very difficult, and a method of alcoholic extraction was used, a weighed amount of the finely ground material being suspended in a Soxhlet extraction shell in a wide-mouth flask attached to a reflex condenser, in such a manner that the condensing alcohol from 50 cc. of boiling alcohol in the bottom of the flask drips through the material to be extracted. The alcoholic extract is titrated. The acids in question are all alcohol soluble. The possibility of error in this method was found to be so great as to render its general use inadvisable. The differences noted in this work however are greater than the possibility of error and are in accord with the expectancies as established by the result of Richards and Spoehr. The results of these acid determinations and those of parallel determinations of total hydrolyzable carbohydrates, are given in table VIII.

A rise in the dry weight in the desiccated joints was noted, which was greatest in those which had been exposed in the open, a rise in acidity in joints desiccating in diffuse light, a drop in acidity in joints desiccating in the open, and a small fall in the hydrolyzable carbohydrate value in desiccating plants, the same for the two conditions of desiccation. The dropping of the carbohydrate figure in the joints desiccating in the open is perhaps to be referred to the fact that the chlorophyllose structure was being slowly affected, for the joints were becoming yellowish, and photosynthetic power was correspondingly lowered. It is rather surprising that the joints which had been resting in weak light for three months still showed at the end of that time so high a content of hydrolyzable carbohydrates. A slow rate of catabolism is apparently indicated.

TABLE VIII

Analyses of desiccating platyopuntias

DESCRIPTION OF MATERIAL	DRY WEIGHT	ACIDITY, CC. $\frac{N}{10}$, NaOH PER G. OF TISSUE	TOTAL HYDROLYZABLE CARBOHYDRATES
	per cent of fresh material		*per cent* of dry wt.
Average of 2 normal joints. Analyzed Jan. 6	12.1	0.83	37.9
Average of 3 joints desiccated indoors 3 mos. Analysed Dec. 31	21.7	1.30	33.1
Average of 3 joints desiccated outdoors 3 mos. Analyzed Dec. 31	25.3	0.52	33.1

The rise in acidity in the plants which had desiccated indoors is probably a result of the long period of low illumination. The drop in acidity in the joints exposed in the open is perhaps a sequence of the falling carbohydrate value. The figures for the two normal joints upon which these assumptions of fall and rise in acidity are made, is fairly typical for plants at the time of day at which the analyses were made, as it is in close agreement with a large number of other observations made in this laboratory.

The hydratation or swelling power of two of the joints was determined at the beginning and at the close of the experiments. Joints no. 7 and no. 19, desiccated outdoors and indoors, respectively, were chosen for this purpose. On September 30 plugs 2 cm. in diameter were cut from each with a cork borer, placed in distilled water in the laboratory equable temperature dark room, and the swelling after twenty-seven hours noted. The wounds healed, and the joints dried out subsequently similarly to the others. On January 7 the experiment was repeated on the same joints. Table IX summarizes the results, imbibed water being measured in per cent of the weight of the imbibing tissue.

Swelling values, not greatly different at the start, diverge widely at the close of the experiment, under the two sets of conditions. Several factors contribute to the change. Chief of these are, osmotic pressure, the amount of dry material involved, and the acidity of the plant sap. From the results of table VIII we may assume that the acidity of no. 7 was higher than that of no. 19, and that the acidity of the latter (desiccated three months in diffuse light) was considerably higher than normal. We know that acidity is an inhibiting factor in water imbibition by the tissues of platyopuntias.

TABLE IX

Hydratation of tissue from desiccating platyopuntias

DESCRIPTION OF MATERIAL	SWELLING, PER CENT OF ORIGINAL WEIGHT	
	Sept. 30	Jan. 7
No. 7, desiccated 3 mos. outdoors. Had lost 24.7 per cent of its original wt.	55.4	84.6
No. 19, desiccated 3 mos. indoors. Had lost 16.8 per cent of its original wt.	51.1	45.6

Accordingly, we are justified in summarizing as follows: nos. 7 and 19 swelled to approximately the same degree on September 30 because they were similar, normal joints. On January 7, no. 7 took up water in vastly greater proportions because it had dried out to a greater extent than no. 19, with the result that the osmotic pressure of its cells was raised, and the relative amount of colloidal material active in hydratation was increased, and also because the acidity of its sap was lower than that of no. 19. It is probably the latter feature, the high acidity, which is responsible for the decrease in swelling power below its normal, observed in no. 19, in spite of three months of desiccation. A slightly lowered sugar content is perhaps an additional factor in this decrease. (Long [15].)

THE EFFECT OF DESICCATION ON THE STRUCTURE OF ECHINOCACTUS WISLIZENI

J. G. BROWN

The effect of desiccation on the structure of living tissues has been studied chiefly by zoologists who were interested in the ability of the rotifers and other invertebrates to survive extended periods of drouth. The results of these investigations, especially those on the cytological changes induced by desiccation in the various organs of rotifers, have been briefly reviewed by Hickernell in a recent paper[9] in which the author also presents the results of his own investigations. Hickernell's paper is especially interesting to botanists for he compares the changes found in tissues of the rotifer with those occurring in plant cells enduring similar dry conditions. He finds that the changes in cell organization in the various tissues of the rotifer are nearly uniform. The cytoplasm shrinks, ceases to exhibit a normal arrangement of its particles, and loses its ability to hold stains. The nucleus is even more sensitive to water starvation. Granular material evidently derived from the large karyosome of the normal nucleus, migrates to the peripheral region of the nucleoplast where it forms a thick ring, so that the relative positions of granular element and ground substance are reversed. No decrease in the size of the nucleus is observable. In the cells recovering from desiccation, nuclei show a reformation of the karyosome and consequent reduction of the granular ring. These changes in the rotifer are compared with those found in cells of desiccated maize embryos and they are found to be analagous. Recovery of desiccated cells in the rotifer is marked by changes almost the reverse of those following drying, according to Hickernell.

Although the periodic drying of plants, such as certain algae, pteridophytes and liverworts, and their subsequent recovery is even more remarkable than the ability of the rotifer to resist desiccation, there is a paucity of literature on the cytological changes in the cells of these plants resulting from conditions of drouth. Pfeffer[10] comments on physiological phenomena attending desiccation, but mentions no changes in cell structure. In this part of the present paper an attempt is made to present a clear picture of the cellular changes in plant tissues, at least so far as the tissues of a succulent are concerned, that result from water starvation.

The chief object of the investigation was to determine changes in structure brought about by continued deprivation of water, and to discover any evidence of recovery on the part of the plant when, after a period of desiccation it was again subjected to normal out-of-door conditions. Preliminary to the study of the desiccated material a careful examination of the structure of the normal plant was made.

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[9] Hickernell, Louis M., A preliminary account of some cytological changes accompanying desiccation. Biol. Bull. **27**: 333–342. 1914.

[10] Pfeffer, W., Physiology of plants, translated by A. J. Ewart. **3**: 249–256. Oxford, 1903.

MATERIAL STUDIED AND ITS PREPARATION

The material used in this study consisted of (1) normal tissue, (2) tissue from the stem of a cactus which had been deprived of water supply for six years, (3) tissue from a plant deprived of water for ten months, and (4) tissue from a plant which had been deprived of water for forty-two months and then transplanted. All of the material with the exception of the normal tissue, was furnished by Dr. D. T. MacDougal of the Desert Laboratory who has given a detailed description of the conditions surrounding the plants from which it was obtained in another section of this publication. This material had been killed and run through the alcohols up to 80 per cent. The normal material was collected by the writer in the neighborhood of the Desert Laboratory, killed in chrom-acetic mixture, and run through the alcohols in the same manner in which the desiccated material had been treated. The usual difficulty in sectioning cactus stems because of the presence of numerous crystals of calcium oxalate was experienced, but sectioning razors were kept sharp, and a fairly good series of slices of each plant was finally obtained. Some of the sections were stained in safranin-orange G, some in safranin Delafield's haematoxylin, some in magdala red; some were treated which chloriodide of zinc and examined while still fresh, and others were treated with iodine. Pieces of the stems studied were taken from apical, middle and basal regions, and comparison was made in each case with tissues from approximately identical locations.

STRUCTURE OF THE NORMAL ECHINOCACTUS

Although the structure of Echinocactus has been described by Schleiden,[11] Preston,[12] and others, it seems advisable to give here a short description of the structure of the normal tissues most directly concerned, in order to facilitate comparison.

The integument consists of a more or less thickened cuticle, an epidermis, with thick external wall and thin vertical and inner tangential walls, and a hypoderm of four or five layers of stone cells with deep pits. The entire integument was found to average 200 micra in thickness in pieces taken 30 cm. below the apex of the stem from parts of ridges half way between the areoles. Of this thickness the cuticle made up 9 micra and the epidermis from 35 to 43 micra. The outer walls of the epidermal cells measured 11 micra in average thickness. The epidermal cells were well supplied with protoplasm and their nuclei measured 6.5 micra in diameter. Stomata had guard cells with anterior walls that averaged 2.0 micra in their thinnest portions and gradually thickened to 3 or 4 micra at their borders near the outer guard cell ridges. Posterior walls of guard cells were 1.2 micra thick. Division of the epidermal cells in a tangential direction results in the formation of new epiderm and hypoderm as described for *Echinocactus eyriesii* by Schleiden. Of the two layers of cells thus formed the lower becomes the phellogen.

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[11] Schleiden, M. J., Beiträge zur Anatomie der Cacteen. Mem. Acad. Sci. St. Petersburg. IV, **4**: 335–380. 1841.

[12] Preston, Carleton E., Southwestern Cactaceae. Bot. Gaz. **30**: 348. 1900. Also **32**: 39. 1901.

Next underneath the hypoderm lies the extensive palisade region, which reaches inward for a distance of 2.5 mm. The cells of the palisade are either elongated cylinders or are barrel-shaped, and are connected at the ends to form long columns. The arrangement is such that the intercellular space is great in extent and the tissue extremely loose, for the cells of each column are in contact laterally with cells of adjacent columns throughout a very small part of their surface area. The shortest cells of the palisade region measured 57 micra in length, the longest ones 275 micra. Single walls were 1 to 2 micra in thickness. All cells were fairly rich in protoplasm considering their size. Chloroplasts were numerous and arranged along the sides of the cells (plate I, fig. 3). Nuclei were lenticular and 32 to 35 micra in length (plate II, fig. 1). The nuclear membrane was thin. In addition to the one nucleolus, numerous granules uniform in size and about one-tenth as large were present. The nucleus was usually located at one side of the cell.

The palisade region of the cortex changes rather abruptl yinward into a tissue composed of rounded cells (plate II, fig, 3), which in the ridges of the stem extends entirely across from one palisade region to another. The cell wall surfaces in contact were here greatly reduced in area, a portion of the cell often extending outward in the form of a short tube to meet a similar process from an adjoining cell. Single walls were from 1 micron or less to about 2 micra in thickness. Plastids appeared occasionally in the cytoplasm. Nuclei were round or oval in form and were 6 to 12 micra in greatest diameter. The cells varied in amount of protoplasmic content, some having only a very delicate network of strands in addition to the layer of cytoplasm around the wall, and others having a much coarser reticulum from wall to wall; none was found in which the protoplasm next to the wall could not be easily recognized with the high power dry lens of the microscope. The location of these cells of the outer cortex and also that of the other regions previously described, is shown in plate I, figure 1.

Deeper cortex consisted of cells mostly rectangular in cross-sections of the stem. These cells varied as much in size as the cells of the outer cortical tissue, but were more compact. The walls were slightly thicker than those of the cortical cells lying near the palisade region, but in amount of protoplasm and in form, size, and structure of the nuclei, the cells of the two regions of the cortex were alike.

In the medulla the cells were mostly irregularly six-sided or rectangular in cross-sections of the stem. They varied in size like the cells of the deep cortex, and had walls 3 to 9.5 micra thick. The cytoplasm varied as in the cortical cells, from a thin sheet closely attached to the walls to a reticulum of loose strands reaching from wall to wall. The nuclei were round or oval in form and about 8 micra in greatest diameter. Their structure was similar to that of the cortical nuclei.

STRUCTURE OF ECHINOCACTUS NO. 7

This plant as previously stated, was without water supply for six years. The integument was about normal in thickness, but the cuticle was thicker than normal, averaging 12 micra. Epidermal cells had external walls 6.4 micra thick, as compared with 11 micra in the normal plant. The nuclei in the epidermal cells were half the size of those in the normal epidermis, averaging only 3 micra in greatest diameter. The cytoplasm formed a thin layer adhering to the external tangential and vertical walls, and a thick layer containing the nucleus on the internal tangential wall. Most of the stomata were partly open; a few had a collapsed appearance. In the latter the cytoplasm of the guard cells was condensed into a small body at the lower side of the cell. Anterior walls of the guard cells averaged 1.3 micra thick in the thinnest portion, and in many cases did not increase much in thickness toward the tangential walls. Posterior walls were 1.6 micra in thickness. Desiccation did not prevent the epidermis from acting in a normal way in the formation of new cork layers (plate I, fig. 2). Epidermal cells observed in the process of division were located in the grooves of the stem. Nuclei in the cells about to divide showed an increase in size as in normal tissue, and the cytoplasm was greater in amount than in the inactive cells of the ridges of the stem.

The palisade region was about 2 mm. thick, 500 micra less than in the normal plant. Many of the cells nearest to the hypoderm, especially those most unsupported by lateral contact with other cells exhibited concave side walls (plate I, fig. 1). Most striking however, was the condition of the protoplasmic contents. In no cell could a layer of cytoplasm be made out entirely around the inside of the cell wall. Every cell examined showed a very small amount of cytoplasm usually restricted to one corner of the cell, in which the nucleus was embedded (plate III, fig. 1). Single cell walls varied from 1.5 to 3.5 micra in thickness. In a few cells the shrunken remains of one or two plastids were visible

The nucleus itself, in the palisade region, was greatly modified in both form and structure. Instead of having the lenticular form found in normal palisade cells, the nucleus was here circular, kidney-shaped, or loaf-shaped. Vacuoles had disappeared from the nucleoplasm and a thickened granular layer had formed in the peripheral portion (plate II, fig. 2). These changes agree with those described by Hickernell for nuclei of dried maize embryos and rotifers. The greatest reduction in size of nuclei was also noticed in the cells of this tract, some nuclei measuring only 4 micra in greatest diameter and the largest 6 to 8 micra, whereas the normal nuclei of the same region measured 32 to 35 micra.

The most conspicuous effect of desiccation on the structure of Echinocactus was the appearance of lacunae in the cortex. When no. 7 was dissected, irregular spaces from a centimeter or two in length to a size just visible to the unaided eye were found by Dr. MacDougal and Mr. Long (plate I, fig. 4). The lacunae appeared in the greater part of the cortex from the outer median region to the vicinity of the stele. Sections supported the suggestion that the spaces were formed by the hydrolysis of cell walls. The earliest stages in the development of lacunae were best shown in sections made from the tissue of Echinocactus no. 25, the specimen desiccated from June to March. In the inner cortex, cell walls were very irregular in thickness and in places presented a swollen appearance (plate III, fig. 2). Application of chloroiodide of zinc gave the characteristic violet cellulose reaction in the denser portions of the walls, but the color gradually faded out in the more gelatinous material of the wall margins. In later stages the walls presented no structural outline, but appeared to merge into the cytoplasm of the cell when the latter was present. Some walls had entirely disappeared (plate III, fig. 4), and others were almost entirely broken down (plate III, fig. 3, *x*). A few cells bordering the lacunae which had not yet grown large, contained cytoplasm. In these cells the nuclei were much shrunken and evidently about to disorganize (plate III, fig. 4 *a*). Cells bordering the larger microscopic lacunae had collapsed so that often four or more walls appeared in sections to form a ropy mass surrounding the lacunar spaces (plate III, fig. 4, *b*).

Vascular bundles of the stele appeared to be unaffected by desiccation. In the medulla, however, the protoplasmic content of the cells was much reduced in amount and the nuclei were smaller than those of the medulla of the normal plant. Cell walls were irregular in thickness and were disintegrating in spots. Several young lacunae were present in most of the sections examined. The effect of the drouth was less marked in the medulla than in the cortex.

STRUCTURE OF ECHINOCACTUS NO. 25

This specimen had been deprived of water for a period of ten months under conditions similar to those of no. 7. The integument showed a normal thickness of about 200 micra. Its cuticular layer averaged 12 micra in thickness. Epidermal cells were mostly normal in appearance, but a few had a reduced amount of cytoplasm, and in the latter the nuclei exhibited the narrow region of thickening in the peripheral portion of the nucleoplasm. Vacuoles had disappeared also and all nuclei of the epiderm were reduced in size, averaging from 3 to 5 micra. Outer epidermal walls in this plant were 12 micra thick. Guard cells were mostly normal, but some had posterior walls thicker than anterior walls.

Palisade tissue was from 1.2 mm. to 2 mm. in thickness. None of the cells presented the shrunken appearance of those in the outer region of the palisade of no. 7. Walls were 1.7 micra thick and were regular in outline. Protoplasm was less abundant than in the normal palisade cells, and chloroplasts only half as numerous. The nuclei were like those of the palisade cells in no. 7, in shape, and were 5 to 7 micra in diameter. Some were non-vacuolated with a narrow, thickened peripheral ring, others were vacuolated.

The outer cortex had the appearance of the corresponding tissue in no. 7, cells being less rounded than normal. Single cell walls were somewhat irregular in thickness, ranging from 1.5 micra to 7 micra. The protoplasm was usually restricted to a layer adhering to the cell wall. Nuclei were 6 to 7 micra in diameter, as compared with 6 to 12 micra in the normal plant.

Median and deep cortex, as previously stated, showed great variation in cell wall thickness, due to swollen parts of walls. The thinnest measured less than 3 micra and the thickest over 20 micra. No lacunae large enough to be seen with the hand lens were found, but a few consisting of the cavities of two or three cells with adjacent intercellular spaces, were observed. Protoplasmic contents of the cells varied in amount, some containing more than the cell shown in plate III, figure 2, others containing as little as the cortical cells of no. 7. A few cells lying close to the phloem of the stele and within 200 micra of cells with swollen walls, contained a varying number of starch grains (plate II, fig. 5). The grains were undergoing digestion, for some were much .corroded and others were already broken into small particles.

Medullary cells were similar to the cells of the deep cortex in irregular thickness of walls. No lacunae were present. Many nuclei exhibited the outer granular ring, and vacuoles had disappeared from the nucleoplasm.

STRUCTURE OF ECHINOCACTUS NO. 6

This plant was allowed to desiccate for forty-two months and was then set in the soil in the open. It was examined twenty-two months later. The integument averaged 240 micra in thickness, the increase over the other specimens being partly due to thicker hypoderm. The cuticle was 12 to 14 micra thick and the outer epidermal wall 10 micra in thickness. Epidermal cells were normal in size and amount of protoplasmic contents, and their nuclei had also become normal in appearance. Some of the stomata had guard cells with posterior walls twice the thickness of the anterior walls, and a few guard cells had anterior and posterior walls of the same thickness.

Palisade cells were normal in shape and in amount of protoplasm. Those near the epidermis exhibited about half as many chloroplasts as were present in the normal cells of the same region, but in no. 6 the chloroplasts were congregated around the nucleus instead of being arranged around the entire side wall. The chloroplasts were also smaller than normal. Nuclei were intermediate in size between those of normal palisade cells and those found in the palisade cells of no. 7. They measured 8 to 12 micra in diameter as compared with 32 to 35 micra in the normal Echinocactus and 4 to 8 micra in plant no. 7. Only one nucleus was observed that had taken on the normal lenticular shape, most of them being round or loaf-shaped like the palisade nuclei of no. 7. The outer thickened ring was reduced in size but still present in a few nuclei, in others it had entirely disappeared. Vacuoles were present in many of the nuclei.

The outer cortex, under low power of the microscope, appeared to be like that of the normal plant, but closer inspection showed that walls were irregular in thickness. The variation in the thickness of the walls ranged from less than 2 micra to over 10 micra. Cell contents also varied from a normal amount to that of desiccated tissue. Nuclei varied from 8 to 13 micra in diameter. Some yet possessed the outer granular ring characteristic of the nuclei of no. 7 and exhibited small vacuoles or none at all; others were normal in structure.

The deeper cortex showed very little evidence of recovery from the effects of desiccation. A few nuclei were larger than normal, measuring from 16 to about 23 micra in diameter, and the cells containing such nuclei had an increased amount of cytoplasm over that of neighboring cells with smaller nuclei. Some of the cells contained no protoplasmic contents that could be discerned with the oil immersion lens. The walls varied in thickness from less than a micron to over 20 micra. Lacunae from the size of two united cell cavities to the size of fifty or sixty cavities were found, but fewer collapsed cells bordered the larger lacunae than were found in no. 7.

No lacunae were found in the medulla, but otherwise this tissue was in a condition similar to that of the inner portion of the cortex.

SUMMARY

A comparison of corresponding regions of the four plants studied is given below.

Integument

NORMAL	NO. 7	NO. 25	NO. 6
200 micra thick	200 micra thick.	200 micra thick.	240 micra thick.
Cuticle 9 micra thick.	Cuticle 12 micra thick	Cuticle 12 micra thick.	Cuticle 12–14 micra thick.
Outer epidermal walls 11 micra thick.	Outer epidermal walls 6.4 micra thick.	Outer epidermal walls 12 micra thick.	Outer epidermal walls 10 micra thick.
Anterior walls of guard cells thicker than posterior walls.	Anterior walls of guard cells thinner than posterior walls.	Anterior walls of guard cells mostly normal but some thinner than posterior walls.	Anterior walls of guard cells normal in some stomata, thinner than posterior walls in others.
Nuclei of epidermal cells 6.5 micra in diameter.	Nuclei of epidermal cells 3 micra in diameter. Thickened granular zone in peripheral portion of nucleoplasm.	Nuclei of epidermal cells 3–5 micra in diameter. Thickened granular peripheral zone in some nuclei.	Nuclei normal.

Palisade

NORMAL	NO. 7	NO. 25	NO. 6
2.5 mm. thick.	2 mm. thick.	1.2–2 mm. thick.	2 mm. thick.
Cytoplasm of cells in layer adhering to wall.	Cytoplasm of cells restricted to one corner of cell.	Cytoplasm of cells in layer adhering to wall.	Cytoplasm of cells in layer adhering to wall.
Chloroplasts numerous, at sides of cells.	No normal chloroplasts.	Chloroplasts reduced to one-half normal number, around sides of cells.	Chloroplasts reduced in number and congregated around nucleus.
Nuclei lenticular, 32–35 micra long, nuclear membrane thin, vacuoles present. Nuclei at side of cell.	Nuclei circular, kidney- or loaf-shaped, 4–6.4 micra long, thickened peripheral layer present, non-vacuolated. Nuclei in corner of cell.	Nuclei like those of no. 7 in shape, 3–7 micra in diameter, narrow peripheral ring present in some which were non-vacuolated. No nuclei at side of cell.	Nuclei mostly like those of no. 7 in shape, 8–12 micra in diameter, outer ring still present in some. Nuclei at sides of cells.

Outer cortex below palisade

NORMAL	NO. 7	NO. 25	NO. 6
Walls of cells 1 micron to 2 micra thick.	Walls 1.5 to 20 micra thick.	Walls 1.5 to 10 micra thick.	Walls 2 to more than 10 micra thick.
Occasional plastids.	No plastids and often no cytoplasm.	A few shrunken plastids around the nucleus.	No plastids.
Nuclei round, 8–12 micra in diameter.	Nuclei, when present round; 5 micra or less in diameter, with thickened peripheral zone.	Nuclei round, 6–7 micra in diameter.	Nuclei round, 8–13 micra in diameter; peripheral zone in some.

Deep cortex

NORMAL	NO. 7	NO. 25	NO. 6
Cell walls 1–3 micra thick.	Cell walls 1 to 25 micra thick.	Cell walls 3 to over 20 micra thick.	Cell walls 1 to over 20 micra thick.
Cells with cytoplasm in layer adhering to wall and in a network, wall to wall.	Little or no cytoplasm.	Cells varied in amount of cytoplasm.	Cells varied in amount of cytoplasm.
Nuclei round, 8–12, micra in diameter.	Nuclei 5 micra in diameter.	Nuclei 4.5–8 micra in diameter.	Nuclei mostly normal; a few 16–23 micra in diameter.
	Large lacunar spaces present.	A few small lacunae found.	Small lacunae found.

Irregularities in thickness of cell walls are the rule in the parenchyma of desiccated Echinocactus stems. They appear to be due to two opposite processes: small irregularities to unequal drying of walls; large irregularities to hydrolysis of walls. The former accounts for the irregular thickness of the walls of palisade cells and the latter for the gross irregularities in the walls of the cortex. The modification in the thickness of the walls of guard cells must seriously affect the activity of stomata. Where anterior walls of guard cells are much thinner than posterior walls it would appear that increased osmotic pressure in the guard cells should cause a closing or partial closing, instead of an opening of the stoma. Eventually, after the continued loss of water by the plant, the guard cells would lose turgidity and slowly collapse, and the stomata would remain permanently open.

Recovery from desiccation is rather slow and is manifest first in the peripheral tissues of the plant; in fact, the deeper parts of the cortex never fully recover from the effects of long-continued drying. In parenchyma cells that have remained alive, the cytoplasm increases in amount, vacuoles appear, and nuclei enlarge and may resume normal form. The granular material that had accumulated in the peripheral portion of the nucleoplasm migrates to the central region of the nucleus. Plastids appear in the region of the nucleus in the palisade cells, and with their formation the plant assumes its normal activity. Incidentally the ability to survive such severe treatment as that to which these Echinocactus plants were subjected clearly demonstrates their admirable adaptation to the arid conditions of their native habitat.

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DESCRIPTION OF PLATES

PLATE I

FIG. 1. Transverse section through outer region of stem of Echinocactus no. 7, from a ridge half way between areoles. *a*, integument; *b*, palisade; *c*, spongy tissue of outer cortex. Oval and circular areas are parts of cell walls coming in contact with adjacent cells. (× 46.)

FIG. 2. Transverse section of cuticle and epidermis of Echinocactus no. 7, showing divided ectoderm cell. The lower cell becomes phellogenistic. (× 572.)

FIG. 3. Normal palisade cell, showing position of nucleus and chloroplasts. (× 1200.)

FIG. 4. Transverse section of lacunar region of the cortex of Echinocactus no. 7. Lacunae are shaded in black. (× 1.)

PLATE II

FIG. 1. Nucleus from normal palisade cell of Echinocactus. Note thin membrane, large nucleolus and vacuoles. (× 1165.)

FIG. 2. Nuclei from palisade of Echinocactus no. 7 showing granular zone. Compare with figure 1. (× 1165.)

FIG. 3. Cells from the cortex of normal Echinocactus, just under the palisade tissue, showing small areas of cell walls in contact and appearance of cell contents. Compare with figure 4. (× 317.)

FIG. 4. Cell from the cortex of Echinocactus no. 7, just under palisade tissue, showing somewhat irregular thickness of walls, restricted area of walls in contact, and reduced protoplast. (× 317.)

FIG. 5. Cell from the deep cortex of Echinocactus No. 25, next to phloem of the stele, showing starch content. (× 273.)

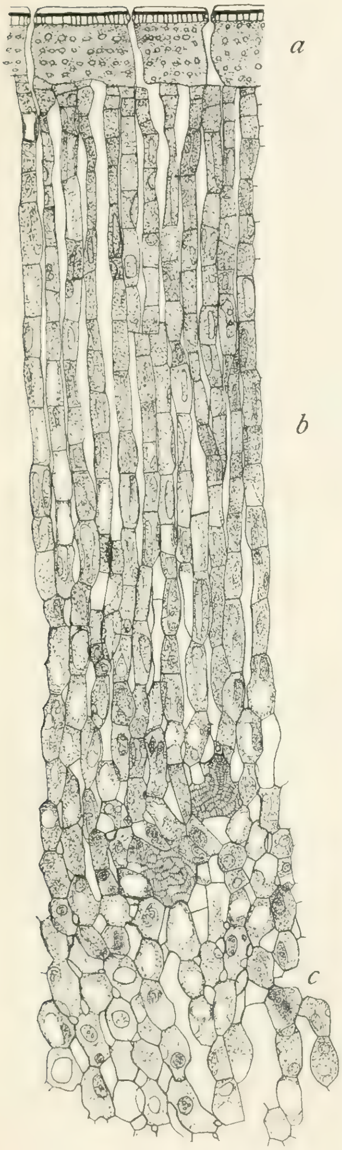
PLATE III

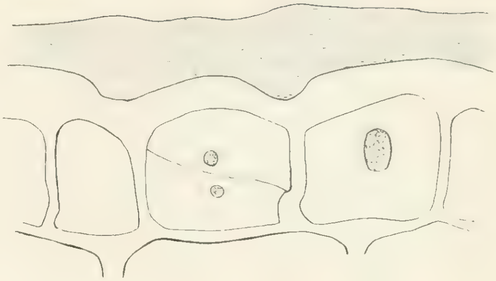
FIG. 1. Palisade cell from Echinocactus no. 7, showing irregular thickness of wall, greatly reduced protoplast, and nucleus with thickened peripheral layer. (× 520.)

FIG. 2. Cortical cell from Echinocactus no. 25, located just outside of phloem, showing irregularities in wall caused by hydrolyzation, and nucleus with granular layer. (× 253.)

FIG. 3. Development of a lacuna in Echinocactus no. 7. *x*, outline of connecting prolongations of two cells: the wall has here almost disappeared. (× 69.)

FIG. 4. Later stage in development of a lacuna in Echinocactus no. 7. A cell containing a disintegrating nucleus is shown at *a*; a mass of gelatinous material resulting from breaking down of several cell walls at *b*. (× 170.)





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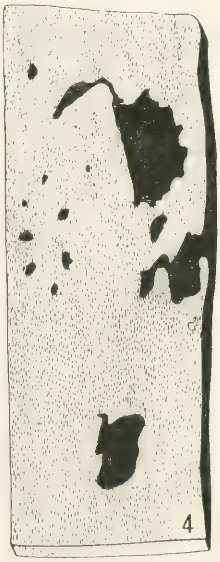

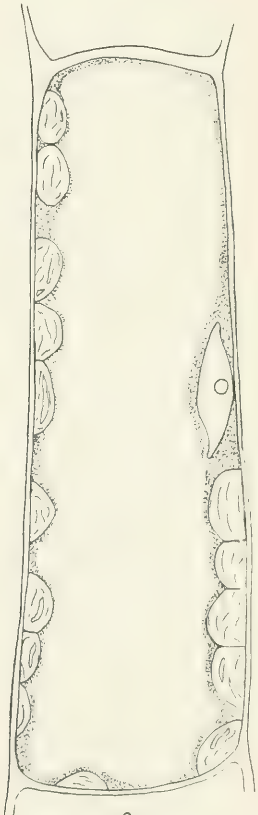

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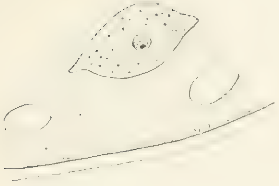

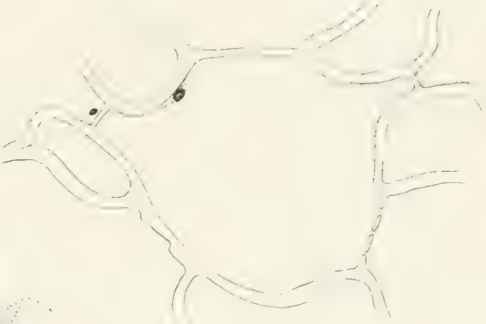

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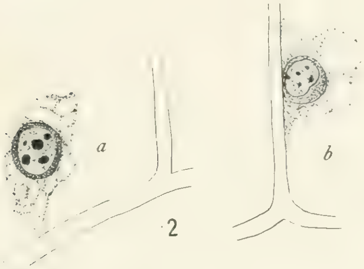

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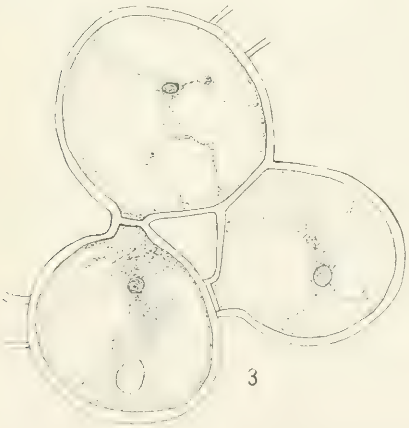

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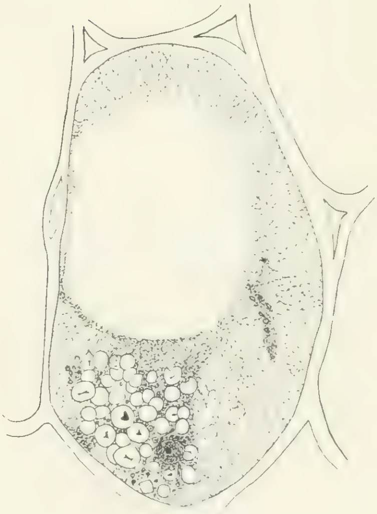

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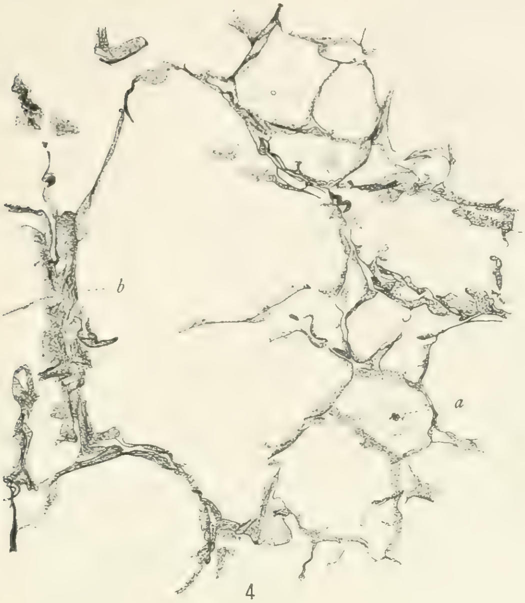

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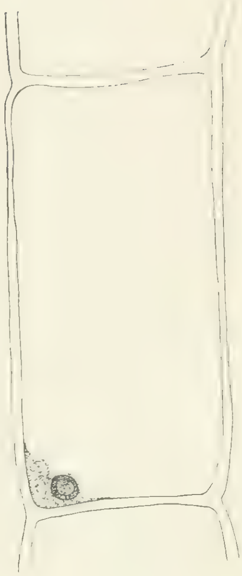

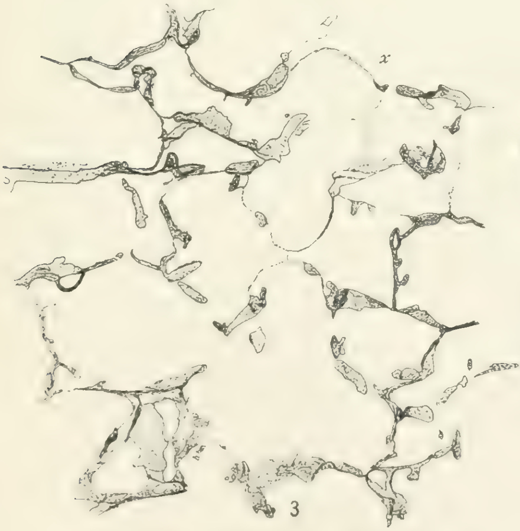

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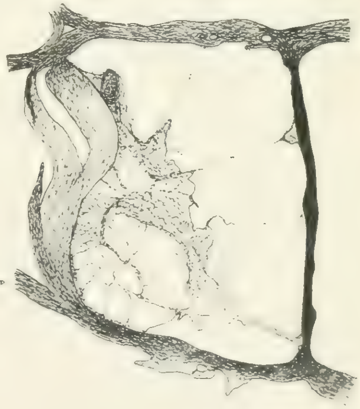

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J. G. Brown: Effect of Desiccation on the Structure of Echinocactus

A STUDY OF PHYSIOLOGICAL BALANCE IN NUTRIENT MEDIA[1]

JOHN W. SHIVE

ABSTRACT[2]

This publication presents the results of an experimental study of the growth of young winter wheat plants in water cultures, with respect to physiological balance of the salts in the medium and to the total concentration of the latter. Three different total concentrations were employed, of 0.1, 1.75, and 4.00 atmospheres, in terms of possible osmotic pressure. For each of these three total concentrations 36 different proportions of the three component nutrient salts, $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4, were simultaneously tested. All possible sets of proportions of these three salts were thus included, for increments of change equal to one-tenth of the total possible osmotic pressure or diffusion tension. A small amount of ferric phosphate was added to each culture solution, to supply iron. There were 36 cultures of 6 plants each, in each concentration series and all three of these series were carried out simultaneously, and then repeated. The culture vessels held 250 cc. and the solutions were renewed every three days, for the total growth period of 23 days which was the same for both triple series. The plants were measured by five quantitative criteria and by two more qualitative ones. The quantitative criteria were: (1) dry yield of tops, (2) dry yield of roots, (3) total water loss by transpiration and guttation during the growth period, (4) water requirement per gram of dry tops, and (5) water requirement per gram of roots. The two more qualitative criteria were: (1) apparent condition of tops, especially as to two forms of leaf injury, and (2) apparent condition of roots, especially as to lateral branching.

The main results of these tests follow.

1. For young wheat plants within the first four weeks of their growth, the three salts here employed form a nutrient medium well suited to the development of the plants, when the total osmotic concentration is about 1.75 atmospheres and when the salts are present in either one of two somewhat similar sets of proportions. One of these two good solutions contains the salts in the proportions: KH_2PO_4, 0.0180 m; $Ca(NO_3)_2$, 0.0052 m; and $MgSO_4$, 0.0150 m. The other has the proportions: KH_2PO_4, 0.0108 m; $Ca(NO_3)_2$, 0.0078 m; and $MgSO_4$, 0.0020 m. In both cases about 0.0044 gram of ferric phosphate is added per liter of solution. These solutions

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[1] Botanical contribution from the Johns Hopkins University, No. 46.

[2] The manuscript of this paper was received July 1, 1915. This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 1, no. 7, October, 1915.

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appear to be nearly equal physiologically; but the first is probably a little better than the second for these plants. The second produced a yield of tops 2 per cent less than the yield given by the first, and a yield of roots 1 per cent less.

2. Judging from the yields of tops and of roots, these solutions are equal to any and are superior to most of the nutrient media previously described by other investigators and now in general use for water culture work, at least when these media are prepared with a total concentration of 1.75 atmospheres.

3. For any given total concentration the best physiological balance of salt proportions for tops is not the same as that for roots.

4. For any given total concentration one set of salt proportions may be found which produces a higher yield of wheat tops than does any other set, and the same is true regarding root yields, but these two sets of salt proportions are different.

5. For any given set of salt proportions the total concentration of the medium determines the growth of the plants. It is quite impossible to select any single set of salt proportions which always produces either very low or very high relative yields throughout the three total concentrations here employed. The relative physiological value of any given set of salt proportions unquestionably varies, in general, with the total concentration of the medium, but no general rule can yet be formulated to express the manner of this variation.

6. The actual values of the top yields, for each set of salt proportions, vary with the total concentrations here employed, but not in the same order; the optimal concentration (1.75 atm.) always gives the highest yield, the supra-optimal (4.00 atm.) gives the medium yield, and the sub-optimal (0.01 atm.) gives the lowest yield.

7. The values of the cation atomic ratios $\frac{\mathrm{Mg}}{\mathrm{Ca}}$, $\frac{\mathrm{Mg}}{\mathrm{K}}$, and $\frac{\mathrm{Ca}}{\mathrm{K}}$ appear to determine the growth of the plants in many cases, but this relation is not always clear for any single ratio; it generally requires two of these ratio values to determine the physiological properties of the nutrient solutions with any given total concentration. The cation atomic proportions characterizing the best physiological balance, as here brought out, vary markedly, and in no simple manner, with the total concentration of the solution.

8. For total concentrations of 1.75 and 4.0 atmospheres serious disturbance of physiological balance manifests itself in more or less pronounced injury to the leaves, the conditions determining this injury being apparently related to the relative proportions of the three salts rather than to the osmotic partial concentration of any one salt.

9. Characteristic pathological conditions in the roots are also observed in those cultures where leaf injury is severe.

10. With total concentrations of 1.75 and 4.00 atmospheres the highest yields of tops were produced by cultures showing slight leaf injury and these same plants usually produced apparently more vigorous root systems than any others of their respective series, but this apparent correlation does not hold, either directly or inversely, for dry yields of roots.

11. The amount of water lost by the plants during the period of their growth appears, in a general way, to give a valuable indication of the top yields, low transpiration generally corresponding to low yields of tops and high transpiration to high yields. This generalization is especially true for the optimal and supra-optimal total concentrations of the medium (1.75 and 4.00 atm.).

12. While no general relation is manifest between the actual amounts of transpiration and the sub-optimal and optimal total concentrations, the culture of the supra-optimal series all agree in giving lower transpiration values than are given by any of the cultures of either the sub-optimal or optimal series.

13. For each set of salt proportions, the water requirement per gram of dry tops varies with the total concentration of the medium, in the reverse order; the water requirement for tops is highest for the sub-optimal concentration and lowest for the supra-optimal.

14. There appears to be no clear general relation between water requirement per gram of roots and total concentration, for the sub-optimal or optimal total concentrations. For almost every set of salt proportions this water requirement is markedly lower, however, for the supra-optimal total concentration than for either the optimal or sub-optimal.

INTRODUCTION

A culture solution may influence a plant growing in it in two ways, corresponding to the two entirely different sets of properties possessed by solutions in general. The chemical properties of the solution may affect the plant in one way, the physical properties in a very different way, and both sets of properties may have an active influence upon the growth of the plant at the same time. Perhaps the physical property of a culture solution most apt to influence plant growth is its osmotic concentration, or diffusion tension, by virtue of which it may tend to resist water absorption by plant roots.

The chemical properties of complex nutrient solutions vary widely according to the nature and proportions of the solutes present. On the other hand, the osmotic properties of a solution containing a given set of solutes in given proportions, in so far as these properties are known to affect plants, are dependent upon the total concentration and upon the extent to which the component solutes are ionized in the solution. The chemical properties and the physical properties may produce the same effect upon an organism, but in very different ways. For example, if the roots of a healthy seedling are plunged into a dilute solution of potassium cyanide, death results almost immediately. It is clear that this is a chemical effect, and that osmotic action can have no influence in bringing about the death of the plant: the solution is of very low osmotic concentration but the solute is highly poisonous. On the other hand, if the roots of such a seedling as that just mentioned be plunged into a molecular solution of cane sugar or glucose, death also results. Here it appears that the injurious action is primarily due to osmotic extraction of water from the roots; it is not due to the entrance of a poison into the organism. When such a non-poisonous solution is not sufficiently concentrated to bring about death through water removal, it may still retard growth and other forms of plant activity, and even bring about death, by hindering water absorption. In such a case the osmotic force resists water entrance instead of causing actual water exit. It is a fact of observation that plants grow less rapidly in more concentrated culture solutions (without toxic effects) than they do in solutions less concentrated but composed of the same salts in the same relative proportions. Changes in growth resulting from alterations in the total concentration of the surrounding medium seem to be dependent upon the ease with which water may be taken into the cells;[3] this is primarily an osmotic relation. A culture solution of high total concentration offers greater resistance to water absorption by plant roots than does a similar solution of lower concentration, and if the medium be sufficiently concentrated to overcome completely the absorbing power of the roots, death of the plant must soon follow from lack of water. Loeb[4] and others have shown that the osmotic extraction of water from an organism by the solution surrounding it may sometimes bring about the same effect as does a chemical change, but chemical and osmotic changes of the medium are not nearly always followed by similar responses.

In studying the chemical influence upon the plant, of the nutrient solution in which it is grown, it is therefore desirable to control, as far as possible, the osmotic influence of the medium as well as its chemical nature. This has been emphasized in the recent work of Tottingham.[5] To vary the chemical nature of the medium in which a plant is rooted, and infer that the resulting physiological reactions are due to the chemical changes in the surroundings, is not logically possible unless the osmotic properties of the solution are known not to have been seriously changed when the chemical properties were altered.

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[3] See in this connection:

Livingston, B. E., On the nature of the stimulus which causes the change of form in polymorphic green Algae. Bot. Gaz. **30**: 289–317. 1900.

——— The rôle of diffusion and osmotic pressure in plants. Chicago, 1903. Pages 124–144.

——— Osmotic pressure and related forces as environmental factors. Plant World **16**: 165–176. 1913.

[4] Loeb, J., On the nature of the process of fertilization and the artificial production of normal larvae (Plutei) from the unfertilized egg of the sea urchin. Amer. Jour. Physiol. **3**: 135–138. 1899.

[5] Tottingham, W. E. A quantitative chemical and physiological study of nutrient solutions for plant cultures. Physiol. Res. **1**: 133–245. 1914.

The general problem of the salt requirements of plants still offers many opportunities for constructive research, and nutrient solutions will surely have to be employed in experiments bearing on this problem, for salts and salt combinations can not be supplied to growing plants excepting in aqueous solution. It therefore follows that nutrient solutions and their effect upon plant growth must be well understood before very satisfactory advances in our knowledge of salt requirements may be expected. Now, in testing the effect of culture solutions upon plants growing in them, it is of course necessary to compare the growth-rate of these plants with that of others grown in some other solution used as a standard of comparison. The use of distilled water as such a standard is generally possible only with very short periods of growth, for plants in distilled water soon become unhealthy and eventually die. Thus while the growth of seedlings in distilled water may be logically compared with their growth in a nutrient solution for the first few days of their growth, it is clearly useless to compare the amounts of growth obtained in these two media during a period of, say, a month; plants in distilled water are frequently dead within two or three weeks and for the remainder of the observation period the dead cultures can furnish no better criterion for growth comparison than can an empty bottle. Even for short growth periods, the use of distilled water as a standard for comparisons has not remained unchallenged. That it is not suitable for such use is obvious, in the first place, because it removes salts from the plants. This phase of the matter has been dealt with by True and Bartlett[6] and by True.[7] Furthermore, as has been shown by many writers,[8] distilled water, unless prepared with exceptional care, appears always to contain small quantities of dissolved poisonous substances, and the resulting toxicity varies in a way not to be readily measured or controlled.

It thus appears that the standard medium required for studies of the salt-nutrition of ordinary plants must be a more or less complex salt solution. This has been somewhat tacitly recognized by many authors, and nutrient solutions for plant cultures have been the subject of many papers in the literature of plant physiology. The results of these studies have been so well brought together and digested in the first part of Tottingham's valuable paper on culture media that only very brief historical consideration is here needed.

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[6] True, R. H., and Bartlett, H. H. Absorption and excretion of salts by roots, as influenced by concentration and composition of culture solutions. U. S. Dept. Agric. Bur. Plant Ind. Bull. 231. 1912.

[7] True, R. H., Harmful action of distilled water, Amer. Jour. Bot. **1**: 255–273. 1914.

[8] For literature in this connection, see:

Livingston, B. E., Further studies on the properties of an unproductive soil. U. S. Dept. Agric. Bur. Soils Bull. 36. 1907.

Hoyt, W. D., Some toxic and antitoxic effects in cultures of Spirogyra. Bull. Torr. Bot. Club **40**: 333–360. 1913.

The first standard nutrient solution for plants was proposed by Sachs[9] in 1860. Since then a large number of different solutions have been recommended as producing good growth of various kinds of plants. Of these, the one most generally employed at the present time is perhaps that of Knop.[10] The medium now generally known as Knop's solution contains by weight four parts of calcium nitrate and one part each of potassium nitrate, magnesium sulphate, and either mono- or di-potassium phosphate.[11] A trace of iron is added.

Tottingham has demonstrated that Knop's solution may be markedly improved for the growth of young wheat plants by altering the proportions of the four main component salts, and it seems probable, or at least possible, that many or all the standard nutrient solutions thus far proposed might be greatly improved by similar modifications. The modified Knop's solution devised by Tottingham gives a considerably greater growth of wheat seedlings than does Knop's solution itself. Certainly, the plan adopted by Tottingham in working out the salt proportions of his nutrient solution, is the most logical and complete method which has appeared in the literature up to the present time, and the solution which he recommends for young wheat plants, based upon such thoroughgoing experimentation as it is, must be regarded as the nearest approach to a standard nutrient medium so far described.

Tottingham employed eighty-four different solutions, all of approximately the same total osmotic concentration, but each solution differing from all the others in its proportions of the four main salts, mono-potassium phosphate, potassium nitrate, calcium nitrate, and magnesium sulphate. Each solution also contained the usual trace of iron, as ferric phosphate, the amount of this salt being the same in all cases. Tests of these eighty-four different sets of proportions of the main nutrient salts, with total osmotic concentrations of 2.50 atmospheres (about the optimum total concentration for young wheat plants) showed that the best solution for the growth of tops during the first four weeks after germination contained the salts in the following volume-molecular proportions: mono-potassium phosphate, 0.0130 m; potassium nitrate, 0.0049 m; calcium nitrate, 0.0144 m; magnesium sulphate, 0.0145 m. This solution showed an improvement of 11 per cent over Knop's solution of the same osmotic concentration, the percentage being calculated on the basis of dry weight of tops grown in the latter solution. The present writer has repeated the tests of Tottingham's optimal series of eighty-four solutions as compared to Knop's solution (all of them having the same osmotic concentration, 2.50 atmospheres), with wheat, and the results are in very satisfactory agreement with those obtained by the earlier author. The best growth of tops (measured in terms of dry weight) occurred with the same proportions of salts as those giving the best growth in the earlier work. While Tottingham reports, for his best solution, an improvement of 11 per cent over Knop's proportions, the corresponding improvement in the repeated experiment was 12 per cent.

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[9] Sachs, J., Vegetationsversuche mit Ausschluss des Bodens über die Nährstoffe und sonstigen Ernahrungsbedingungen von Mais, Bohnen und anderen Pflanzen. Landw. Versuchsst. **2:** 219–268. 1860.

[10] Knop, W., Quantitative-analytische Arbeiten über den Ernährungsprocess der Pflanzen. II. Landw. Versuchsst. **4:** 173–187. 1862.

[11] The di-potassium salt seems to be more frequently met with in the literature than is the mono-potassium salt, in connection with Knop's solution, but the latter is by far the more satisfactory of the two, on both chemical and physiological grounds, as has been clearly and convincingly shown by Tottingham.

From a consideration of Tottingham's work, as well as from earlier publications, it is clear that nutrient solutions prepared according to the formulas of Knop and Tottingham are very suitable for supporting plant growth, and Tottingham's solution may safely be employed as a standard for culture comparisons. However, in all experimentation bearing on the growth of plants in nutrient solutions, and related to the general problems of salt requirements, physiological balance, and salt antagonism, it is quite essential that the standard nutrient solution used as a basis of comparison be as simple as possible, besides being capable of producing excellent growth. Whatever salts the standard solution may contain, it should be characterized by approximately optimum salt proportions and, at the same time, should have approximately optimum osmotic concentration for the particular kind of plant upon which the experimentation is to be carried out. Since it may be supposed that different plant forms may differ in their requirements for a physiologically balanced solution, it is clear that such a chemically and physically optimum solution can not be satisfactorily determined by the usual hit-or-miss procedure in such cases (calling that *optimum* which has been shown only to be *good*), but must rest upon a more or less thorough testing of a rather large number of possibilities. Tottingham's general method for finding the optimum solution for a given set of salts and for a given plant is the only one available, and, although it may appear somewhat elaborate and tedious in operation, yet its employment is quite essential if the physiological properties of the medium in which growth is to be taken as standard are to be at all well understood. It thus appears that studies on salt nutrition will frequently have to involve some such series of tests as those employed by Tottingham. In such work, the simpler the mixture may be, the more readily may its optimum, as regards salt proportions and osmotic concentration, be determined. If a simple series of salts may be so brought together as to give as good plant growth as does the optimal mixture of a more complex series, the former is much to be preferred.

The experimentation here to be set forth aimed primarily to find out whether a simpler solution than that used by Tottingham might not be possible, without loss of ability to support plant growth. Possible simplification lies here only in the employment of a smaller number of salts. Tottingham used four main salts in eighty-four different combinations for each osmotic concentration tested. If the number of salts might be reduced to three, a similar series of tests (increments of partial concentrations being, as in Tottingham's work, one-tenth of the total concentration), would involve only thirty-six different combinations, which would entail a saving of work greatly to the advantage of rapid progress. If a two-salt solution might be employed, such a series of tests would include but nine combinations, but such a solution is clearly impossible in the present case, since six different chemical elements (K, Ca, Mg, N, S, and P) are required in every adequate nutrient medium, and only two of these can be carried by a single salt. It appears, then, that a three-salt mixture (neglecting the trace of iron, which is of course always to be added) is logically possible as a standard culture solution, and that such a standard solution containing but two salts is impossible on *a priori* grounds.

The omission of potassium nitrate from the Knop-Tottingham formula leaves the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulphate, which, with the trace of ferric phosphate also present, contain all the essential elements required in a nutrient solution. These dissociate in solution to form all the ions present in the Knop-Tottingham four-salt solution. They do not precipitate too readily when mixed in solution, and they permit total concentrations suitable for plant growth. Such a three-salt solution seems to be chemically as well adapted to the growth of plants in water culture as is the Knop-Tottingham four-salt solution.

It was the purpose of this investigation to study the properties and possibilities of the three-salt combination just suggested, and to determine, as nearly as possible, the salt proportions needed to produce approximately optimal growth of wheat and of buckwheat, during early stages of their development. The results obtained with wheat will alone be presented in the present paper; those for buckwheat will be published later.[11a]

The investigation was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University. It is a pleasure to acknowledge indebtedness to Professor Burton E. Livingston, not only for suggesting the problem, but also for helpful advice during the progress of the work, and for valued aid and criticism in the preparation of the manuscript.

MATERIALS AND METHODS

THE STOCK SOLUTIONS OF SINGLE SALTS

The salts used in the present work were Merck's "highest purity" mono-potassium phosphate and calcium nitrate, and Merck's "blue label" magnesium sulphate, the last containing the usual seven molecules of water.

[11a] A preliminary report of some of the results of the present study has already appeared:—Shive, J. W., A three-salt nutrient solution for plants. Amer. Jour. Bot. **2** : 157-160. 1915.

It was found necessary to dry the calcium nitrate, because of varying amounts of water in the lumps of salt. This was accomplished by heating the salt in a large crystallizing dish in an electric oven at 150°C., until it formed a solid mass. It was then pulverized in a mortar, returned to the oven, and dried to constant weight at 150°C. Any free moisture contained in the stock supply of the other salts was eliminated by drying them at 105°C. and allowing them to cool in a desiccator, from which they were taken as needed.

In preparing the original stock solutions, from which, after further dilution, the culture solutions were to be made up, gram-molecular weights of the salts (weighed to one milligram) were dissolved separately in Jena glass flasks, and each solution was then made up to liter volume at 15°C. These solutions were always stored in the flasks in which they were prepared, but were never kept for a long time; in no case longer than six days. The distilled water used throughout was obtained from a "Barnstead" still, with tin-lined condenser, and was stored in a twenty-gallon stone-ware jar, from which it was drawn through a block tin tube into a glass carboy for immediate use. Throughout this work, special precautions were taken to clean thoroughly (with potassium bichromate in sulphuric acid followed by thorough rinsing) all vessels employed as containers of distilled water or of solutions.

For immediate use the volume-molecular single salt solutions were diluted to concentrations better suited to the preparation of the culture media themselves. These diluted stock solutions were kept in liter flasks. Each of these flasks was connected with the upper end of a 50 cc. burette, graduated to 0.1 cc. and provided with a glass stopcock below. The connection was made by means of a glass tube bent into the form of an inverted U, one end extending to the bottom of the flask, the other passing through a tightly fitting stopper in the top of the burette and extending downward just to the zero mark on the burette scale. A second glass tube was also passed through the stopper in the top of the burette, and ended just below the lower surface of this stopper. Suction applied to this tube caused the solution to flow from the flask into the burette, thus filling the latter. Since the longer arm of the U-tube extended to the bottom of the flask, and since the zero of the burette scale was always at a higher level than the surface of the liquid in the flask, any excess of solution in the burette siphoned back into the flask when suction was discontinued, leaving the burette filled to the zero mark. In this way the solutions came into contact with glass surfaces only, and this device for automatically obtaining the proper level in the burette after each measurement was found to be a valuable saver of time. The concentrations of these diluted stock solutions were so calculated that no less than 2 cc. of any one was ever drawn from the burette at one time, in the preparation of the culture solutions; errors that might result from slight inaccuracies in reading burettes graduated only to 0.1 cc. were thus largely obviated.

THE CULTURE SOLUTIONS

In a series of solutions having three given constituent salts, the relative proportions of these three salts may, of course, be varied so as to give a very large number of solutions, all of the same total concentration; and the total concentration of a culture solution with any given set of proportions of the three salts may likewise be varied to give another large number of solutions, differing neither in the kind of salts nor in their relative proportions, but only in the total concentration.

Throughout this paper, concentrations will be expressed in terms of diffusion tension or maximum osmotic pressure (osmotic concentration), in atmospheres, or in terms of gram-molecules per liter of solution (volume-molecular concentration).

Three series of nutrient solutions were employed, each solution containing the three salts, mono-potassium phosphate, calcium nitrate, and magnesium sulphate, in varying proportions. These three series differed widely in total concentration, and were so chosen that one possessed a total concentration about optimal for wheat as here grown, one of the others being below, and one above this total concentration. All solutions of the same series had approximately the same osmotic concentration. Tottingham called attention to the fact that the concentration employed for his optimal series (2.50 atmospheres of diffusion tension) may be slightly above that required for optimal growth of wheat, and the results of preliminary experiments with three-salt mixtures, in series such as are to be described below, showed that a total salt concentration of 1.75 atmospheres is well within the range required for optimal growth of wheat. These tests showed further that a concentration or 4.0 atmospheres is well above the optimal range for wheat. The following experiments were therefore carried out with three series of nutrient solutions having concentrations of 0.1, 1.75, and 4.0 atmospheres, the first being chosen as having been shown by preliminary tests to lie well below the optimal range of total concentration for the plant here employed. These three series will hereafter be termed sub-optimal (0.1 atm.), optimal (1.75 atm.), and supra-optimal (4.0 atm.).

The method here adopted to control the osmotic concentrations in a series of solutions differing in the proportions of the three salts used was the same as that employed by Tottingham. In varying the relative proportions of the component salts apportionments were made in such a way that a decrease in the partial concentration of one salt was balanced by increases in the partial concentrations of the remaining salts, sufficient to keep the total osmotic concentration of the solution constant. The total osmotic concentration to be employed for any series was considered to be divided into ten equal parts and these parts were distributed among the three salts in all possible proportions. Thus each salt produced, in the different solutions of the same series, from one to eight-tenths of the total osmotic concentration.

A detailed discussion of the methods of calculation by which the partial diffusion tension and volume-molecular concentration of each salt, for such complex mixtures with a fixed total osmotic concentration, may be approximately determined, has been presented by Tottingham (pp. 177–182 and 192) and need not be repeated here. The calculations were carried out in such a manner as to allow every three-salt solution to contain that particular amount of each salt which would have been required to produce the desired partial osmotic concentration if that salt had been alone in the solution. Each solution contained an amount of each of the three salts such that the total osmotic concentration should possess the fixed magnitude required by the particular series to which the solution belonged, if it were assumed that the presence of the remaining two salts exerted no influence upon its degree of ionization. Expressed in another way, each of the three salts may be conceived as placed in the solution of the other two as though that solution were pure water, so as to give the requisite osmotic concentration for the first salt (one, two, etc., tenths of the total diffusion tension of the complete solution) without any reference to the presence of the other two.

The lowering of the freezing-point was determined for all of the three-salt solutions here employed, and the osmotic concentration was calculated from the lowering in each case. The method here used was the same as that described by the writer in connection with his study of Tottingham's solutions.[12] From these determinations it appeared that the lowerings were approximately the same for all of the complete solutions of the sub-optimal and optimal series. It therefore follows, in these cases, from the relation holding between the lowering of the freezing-point and maximum possible osmotic pressure, that the amount of salt required to produce one-tenth of the total osmotic concentration of the complete solution was also constant for the sub-optimal and for the optimal series. This amount may be termed the osmotic unit for the series in question, following Tottingham's similar usage. In the supra-optimal series, however, the value of this osmotic unit was variable, becoming greater or less in magnitude as the partial osmotic concentration of the salt in question was increased or decreased. This, of course, is to be expected in such solutions as these, where the concentration of the salt is too high to permit approximately complete dissociation.

The volume-molecular partial concentrations of the solutions in the supra-optimal series were calculated to give a total osmotic concentration of 4.50 atmospheres, but the cryoscopic tests gave depressions of the freezing-point indicating a total osmotic concentration of only about 4.0 atmospheres in every case, throughout the entire series. This seems to indicate that the decrease in the degree of dissociation caused by the influence of one salt upon another is considerable in these more concentrated mixtures. With the lower concentrations, sub-optimal and optimal, this influence is lacking, or is too slight to be detected by the methods here employed. Tottingham's supra-optimal series of nutrient solutions showed a similar depression in total osmotic concentration when this was determined by the cryoscopic method (Shive [1914]). The average diffusion tension of that entire series was 7.22 atmospheres, while Tottingham's calculation placed this value at 8.15 atmospheres.

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[12] Shive, J. W., The freezing points of Tottingham's nutrient solutions. Plant World 17: 345–353. 1914. In table I, page 349, of this paper, the second number in the fifth column should be 2.53 instead of 2.40 as given.

The subject of dissociation equilibrium in solutions of two salts possessing a common ion is treated in text-books of physical chemistry, but the influence of one salt upon the dissociation of others in complex solutions where no common ion is present has not yet been worked out satisfactorily. Mellor[13] points out that there is no interchange of ions in mixed solutions of strongly dissociated electrolytes, but that the solutions contain the same ions as were previously present in the unmixed solutions. With feebly dissociated electrolytes the phenomenon is much more complex, and is not yet understood well enough to allow satisfactory calculation.

The results of the cryoscopic tests justify the supposition that all the solutions of the sub-optimal series possessed a total osmotic concentration of 0.1 atmosphere, and, similarly, that those of the optimal series had a total concentration of 1.75 atmospheres, within the limits of accuracy imposed by the cryoscopic method as here employed. Since the solutions of the supra-optimal series agreed in showing a lowering of the freezing-point corresponding to a diffusion tension of 4.0 atmospheres, instead of 4.50 as calculated, it is safely to be supposed that these solutions actually exhibited an osmotic concentration very close to 4.0 atmospheres. In default of more precise knowledge, these suppositions will be regarded as true in the following discussions. Whatever may be the magnitude of the errors thus involved, it is certain that such variations from the assumed osmotic concentration can not be great enough in any case to produce any sensible influence upon the plants; such organisms are much less sensitive to osmotic conditions of the medium than is the cryoscopic method of determination as here used.

Table I gives the volume-molecular partial concentrations of each salt required to produce from one-tenth to eight-tenths of the total osmotic concentration, for each of the three series of solutions here considered. To determine the volume-molecular partial concentration of any salt in any solution of the three series, it is necessary simply to find in the first column the number indicating how many tenths of the total osmotic concentration are to be apportioned to the particular salt in question, and then to read the required partial volume-molecular concentration, given opposite this number on the same line, in the proper column for the particular series and salt involved.

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[13] Mellor, J. W., Chemical statics and dynamics. London, 1909. Pages 202–205.

For convenience in the preparation of the three series of complete nutrient solutions to be described later, the concentrations of the stock solutions from which the culture solutions were prepared differed widely in the three series, but the three separate stock solutions used in the preparation of each single series were equal in molecular concentration. The concentrations of the stock solutions used in the preparation of the sub-optimal, optimal, and supra-optimal series were one-fortieth volume-molecular (m/40), one-fourth volume-molecular (m/4), and volume-molecular (m), respectively.

In preparing a culture solution, the required amount of stock solution of mono-potassium phosphate was first drawn from the proper burette into a 250 cc. volumetric flask about three-fourths filled with water. To this was now added, in a similar manner, the required amounts of the stock solutions of calcium nitrate and of magnesium sulphate, in the order here given. As the solutions were added, the flask was shaken to promote mixing. The volumetric flask was finally filled to the mark with water.

TABLE I

Volume-molecular partial concentrations of mono-potassium phosphate, calcium nitrate, and magnesium sulphate required to produce from one to eight tenths of the total osmotic concentration, for each of the three series of solutions

TENTHS OF TOTAL CONCENTRATION	SUB-OPTIMAL SERIES (TOTAL CONCENTRATION 0.1 ATM.)			OPTIMAL SERIES (TOTAL CONCENTRATION 1.75 ATM.)			SUPRA-OPTIMAL SERIES (TOTAL CONCENTRATION 4.0 ATM.)		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
1	0.0002	0.00015	0.0003	0.0036	0.0026	0.0050	0.0090	0.0066	0.0142
2	0.0004	0.00030	0.0006	0.0072	0.0052	0.0100	0.0180	0.0132	0.0258
3	0.0006	0.00045	0.0009	0.0108	0.0078	0.0150	0.0270	0.0189	0.0428
4	0.0008	0.00059	0.0011	0.0144	0.0104	0.0200	0.0368	0.0265	0.0570
5	0.0010	0.00074	0.0014	0.0180	0.0130	0.0250	0.0460	0.0331	0.0713
6	0.0012	0.00089	0.0017	0.0216	0.0156	0.0300	0.0580	0.0417	0.0890
7	0.0014	0.00104	0.0020	0.0252	0.0182	0.0350	0.0690	0.0496	0.1070
8	0.0017	0.00119	0.0023	0.0288	0.0208	0.0400	0.0800	0.0576	0.1240

To each complete solution, as it was prepared, was added three drops of a uniform suspension of ferric phosphate in distilled water. Each cubic centimeter of this suspension contained approximately 0.0022 g. of ferric phosphate, or 0.00082 g. of iron. The ferric phosphate was prepared by precipitation from a solution of ferric nitrate with mono-potassium phosphate, and the precipitate was subsequently thoroughly washed.

Variations in the proportions of the three salts here employed, by increments of one-tenth of the total osmotic concentration, produce a series of thirty-six solutions, as has been remarked. Similar variations in the four salts used by Tottingham yield a series of eighty-four solutions. The smaller number of solutions is a distinct advantage in favor of a three-salt medium,—

an advantage that can scarcely be overestimated, considering the large number of cultures to be dealt with in studies of this kind.

For the concrete expression of the relative osmotic proportions of the three salts in each solution of each series, for facility in designating individual cultures, and for ease of reference in discussion, the thirty-six solutions may be conveniently arranged on a triangular diagram, as shown in figure 1. Graphic schemes similar to this have been used extensively in physical chemistry, for considerations involving relative proportions of three component parts. The diagram as here used was employed by Schreiner and Skinner[14] in a study somewhat similar to the present one. Each side of an equilateral triangle is divided into seven equal parts, and lines are drawn through each of the points of division thus obtained, parallel to each of the other two sides of the triangle. Each of the original points and each of the intersections of the lines represents a culture solution or a culture. The diagram shows eight horizontal rows of cultures, one above the other. The lowest row contains eight cultures, the next seven, etc., and the eighth row contains but a single one. All the solutions represented in the lowest row have approximately one-tenth of their total osmotic concentration due to mono-potassium phosphate, those in the second row have approximately two-tenths due to this salt, and so on, until the apex of the triangle is reached. This culture, representing the eighth row, has approximately eight-tenths of its total

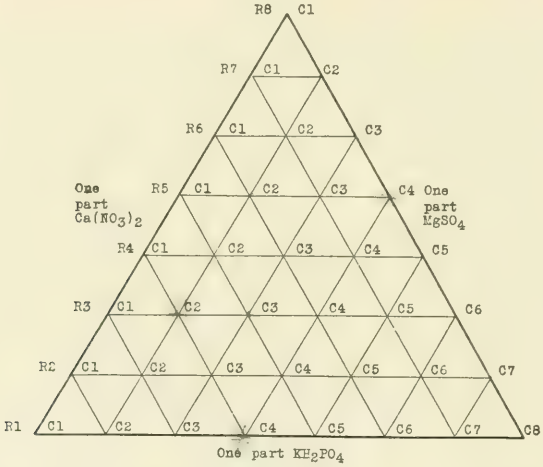

FIG. 1. Diagram showing solution numbers and osmotic proportions of the three salts.

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[14] Schreiner, O. and Skinner, J. J., Ratio of phosphate, nitrate, and potassium on absorption and growth, Bot. Gaz. **50:** 1–30. 1910.

osmotic concentration due to mono-potassium phosphate. The first culture at the left of each row has one-tenth of its total osmotic concentration due to calcium nitrate, the second culture of each row has two-tenths derived from this salt, and so on to the opposite side of the triangle. Thus the eight cultures of the first row have, respectively, from one-tenth to eight-tenths of their total osmotic concentration due to the calcium salt. The seven cultures of the second row have from one-tenth to seven-tenths of their osmotic concentration derived from the calcium salt, and so on to the apex of the triangle. The last culture of each row has one-tenth of its total osmotic concentration derived from magnesium sulphate. Proceeding from the right to the left side of the triangle, the partial osmotic concentration of magnesium sulphate in each solution increases in a manner similar to that followed for calcium nitrate passing in the opposite direction. Thus the eight cultures of the first row have from one-tenth to eight-tenths of their osmotic concentration due to calcium nitrate, and from eight-tenths to one-tenth produced by magnesium sulphate, and in the cultures of each succeeding row, the osmotic proportions of these two salts are similarly distributed. Each side of the diagram may be designated by the name of one of the three salts; thus the base (as shown in fig. 1) may be considered as the mono-potassium side, while the left and right sides are, respectively, the calcium nitrate and the magnesium sulphate sides.

The method adopted by Tottingham (p. 194) to designate individual cultures is again employed here. The rows of cultures are numbered R1 to R8, from base to apex of the triangle, and the individual cultures are numbered in each row from left to right, C1 to C8. Thus the cultures of the first row are numbered R1C1 to R1C8, the cultures of the second row, R2C1 to R2C7, etc., until the single culture of the eighth row is numbered R8C1. To determine the osmotic proportions of the salts in any culture solution, it is only necessary to observe the row in which it occurs and its number in the row. Thus, culture R4C2 has four-tenths of its osmotic concentration due to mono-potassium phosphate, since it is in row four; two-tenths due to calcium nitrate, since it is culture number two from the left side; and four-tenths derived from magnesium sulphate, since it is the fourth culture from the right side of the triangle.

In table II are given the actual partial volume-molecular concentrations of each solution of the three series used in the present study. The solution numbers in the table correspond to the numbers in the diagram of figure 1, already explained.

It will be observed that solutions R1C3 to R1C8, also R2C4, R2C5, and R2C7, of the supra-optimal series, are omitted from this table. These solutions formed slight precipitates after standing from one to three days, and were rejected on that account, as not suitable for a study of this kind.

TABLE II

Partial volume-molecular concentrations of mono-potassium phosphate, calcium nitrate, and magnesium sulphate in the solutions employed in the sub-optimal, optimal, and supra-optimal series.

SOLUTION NUMBER	PARTIAL CONCENTRATION, VOLUME-MOLECULAR								
	Sub-optimal series (0.1 atm.)			Optimal series (1.75 atm.)			Supra-optimal series (4.00 atm.)*		
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$
R1C1	0.00021	0.00015	0.00228	0.0036	0.0026	0.0400	0.0090	0.0066	0.1240
C2	0.00021	0.00030	0.00199	0.0036	0.0052	0.0350	0.0090	0.0132	0.1070
C3	0.00021	0.00045	0.00171	0.0036	0.0078	0.0300	....	....	....
C4	0.00021	0.00059	0.00124	0.0036	0.0104	0.0250	....	....	....
C5	0.00021	0.00074	0.00114	0.0036	0.0130	0.0200	....	....	....
C6	0.00021	0.00089	0.00086	0.0036	0.0156	0.0150	....	....	....
C7	0.00021	0.00104	0.00057	0.0036	0.0182	0.0100	....	....	....
C8	0.00021	0.00119	0.00028	00.036	0.0208	0.0050	0.0090	0.0576	0.0142
R2C1	0.00041	0.00015	0.00199	0.0072	0.0026	0.0350	0.0180	0.0066	0.1070
C2	0.00041	0.00030	0.00171	0.0072	0.0052	0.0300	0.0180	0.0132	0.0890
C3	0.00041	0.00045	0.00142	0.0072	0.0078	0.0250	0.0180	0.0198	0.0713
C4	0.00041	0.00059	0.00114	0.0072	0.0104	0.0200	....	....	....
C5	0.00041	0.00074	0.00086	0.0072	0.0130	0.0150	....	....	....
C6	0.00041	0.00089	0.00057	0.0072	0.0156	0.0100	0.0180	0.0417	0.0285
C7	0.00041	0.00104	0.00028	0.0072	0.0182	0.0050	....	....	....
R3C1	0.00062	0.00015	0.00171	0.0108	0.0026	0.0300	0.0270	0.0066	0.0890
C2	0.00062	0.00030	0.00142	0.0108	0.0052	0.0250	0.0270	0.0132	0.0713
C3	0.00062	0.00045	0.00114	0.0108	0.0078	0.0200	0.0270	0.0198	0.0570
C4	0.00062	0.00059	0.00086	0.0108	0.0104	0.0150	0.0270	0.0265	0.0428
C5	0.00062	0.00074	0.00057	0.0108	0.0130	0.0100	0.0270	0.0331	0.0285
C6	0.00062	0.00089	0.00028	0.0108	0.0156	0.0050	0.0270	0.0417	0.0142
R4C1	0.00082	0.00015	0.00142	0.0144	0.0026	0.0250	0.0368	0.0066	0.0713
C2	0.00082	0.00030	0.00114	0.0144	0.0052	0.0200	0.0368	0.0132	0.0570
C3	0.00082	0.00045	0.00086	0.0144	0.0078	0.0150	0.0368	0.0198	0.0428
C4	0.00082	0.00059	0.00057	0.0144	0.0104	0.0100	0.0368	0.0265	0.0285
C5	0.00082	0.00074	0.00028	0.0144	0.0130	0.0050	0.0368	0.0331	0.0142
R5C1	0.00103	0.00015	0.00114	0.0180	0.0026	0.0200	0.0460	0.0066	0.0570
C2	0.00103	0.00030	0.00086	0.0180	0.0052	0.0150	0.0460	0.0132	0.0428
C3	0.00103	0.00045	0.00057	0.0180	0.0078	0.0100	0.0460	0.0198	0.0285
C4	0.00103	0.00059	0.00028	0.0180	0.0104	0.0050	0.0460	0.0265	0.0142
R6C1	0.00123	0.00015	0.00086	0.0216	0.0026	0.0150	0.0580	0.0066	0.0428
C2	0.00123	0.00030	0.00057	0.0216	0.0052	0.0100	0.0580	0.0132	0.0285
C3	0.00123	0.00045	0.00028	0.0216	0.0078	0.0050	0.0580	0.0198	0.0142
R7C1	0.00144	0.00015	0.00057	0.0252	0.0026	0.0100	0.0690	0.0066	0.0285
C2	0.00144	0.00030	0.00028	0.0252	0.0052	0.0050	0.0690	0.0132	0.0142
R8C1	0.00165	0.00015	0.00028	0.0288	0.0026	0.0050	0.0800	0.0066	0.0142

* Eight solutions of the supra-optimal series formed precipitates after standing from one to three days and are for this reason omitted from the table.

THE PLANTS AND THEIR TREATMENT

The wheat used was of the variety known as Fulcaster, from the same lot as was used by Tottingham in 1913, which was supplied by the United States Department of Agriculture. All the seed used was fairly uniform in size, and germinated well.

Germination was brought about by a modification of the methods employed by Livingston,[15] by Schreiner and Skinner,[16] by other workers in the United States Bureau of Soils, and by Tottingham. The seeds were soaked in distilled water for a period of from six to eight hours and were then placed on wet blotting paper in a moist chamber for from twelve to fifteen hours, or until they germinated. Uniform and vigorously germinated seeds were then selected, one by one, from the lot, and were placed upon the germination net, to grow till large enough for the culture bottles.

This net is constructed as follows: A glass rod, 5 mm. in diameter, is bent to form a square frame, with one end of the rod continued to form a diagonal across the square. This frame is placed between two layers of paraffined mosquito netting, and the whole rests upon the top of a circular "graniteware" pan (35 cm. in diameter and 7 cm. deep) with the corners of the frame resting on the slightly projecting rim. The two pieces of netting are firmly stretched over the top of the pan and over the frame by binding beneath the marginal rim of the pan with a heavy cord. The top layer of netting upon which the germinating seeds are to be placed, is thus raised about 5 mm. above the top of the pan, while the lower net is nearly parallel to the upper and about 5 mm. below it. While water is allowed to flow into the pan and overflow, the water surface comes into contact with the upper net, but does not completely flood the seeds, which are always in contact with water, and still freely exposed to the atmosphere.

The pan with the germinating seeds stood in a greenhouse room, and a stream of tap water was allowed to flow into the pan continuously, through a small-bore glass tube that penetrated the double net. After three or four days, this procedure resulted in very uniform and vigorous seedlings about 5.5 cm. tall and entirely free from fungus. Flowing tap water seemed to yield better results than could be obtained by the use of distilled water, even with frequent changes, as was, indeed, to be expected, on account of the extraction or leaching effect always produced by distilled water. The double net is necessary for buckwheat, but the lower one may be omitted for wheat.

Selected seedlings, from 5 to 6 cm. tall, were mounted in thoroughly paraffined, flat cork stoppers (in a manner similar to that described by Tottingham, pages 173–175), which were then placed in the culture bottles. The latter were wide-mouth flint-glass bottles of 250 cc. capacity. These had been in use as culture vessels for several years, so that the absence of excessive amounts of soluble matter at the inner surface of the glass was assured.

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[15] Livingston, B. E., A simple method for experiments with water cultures. Plant World 9: 13–16. 1906.

[16] Schreiner, O. and Skinner, J. J., Some effects of a harmful organic soil constituent. U. S. Dept. Agric. Bur. Soils Bull. 70. 1910.

In water culture work, the glass culture vessels are sometimes covered with black paper or other black material, to exclude light from the roots of the plants. This practice is particularly objectionable when cultures are exposed to direct sunlight, since the rate of heat absorption by the black material is often sufficient to raise the temperature of the culture solutions considerably above that of the surroundings—a complication to be avoided as far as possible. In the present work, the culture vessels were covered with shells or jackets of Bristol board, which was dark brown on one side and nearly white on the other. To make these shells, rectangular pieces of Bristol board were cut sufficiently long to wrap around the culture bottle, with some overlapping; the pieces were about 1.5 cm. greater in width than the height of the bottle. One long edge of this rectangle was folded over so as to form a projection about 1.5 cm. wide, perpendicular to the remaining part. This projecting portion was slashed across from its free edge, at intervals of about 1.5 cm., so as to allow the cut edges to overlap when the main portion was wrapped about the culture bottle. When this cylindrical shell, which was dark within and almost white without, was in place, the horizontal upper rim slightly overlapped the edge of the cork stopper in the mouth of the bottle. Light was thus almost completely excluded from the roots of the seedlings, and undue absorption of heat was avoided. These shells were held in place by a cord fastened around the outside; they could be easily and quickly removed from the culture bottles at any time, and replaced again without disturbing the cultures. With proper care, such shells may be used repeatedly.

The seedlings were mounted in the cork stoppers and placed in the culture bottles as rapidly and as carefully as possible, so that effects incident to handling should be approximately the same on all the seedlings, and the shock of transfer should be reduced to a minimum.

In studying the physiological effects of nutrient solutions it is always recognized as desirable to control all conditions affecting the organisms excepting those directly dependent upon the properties of the solutions, but this has never yet been satisfactorily accomplished. If aerial conditions can not be kept constant, it is clearly essential that all the cultures of a series be exposed to these conditions in an approximately similar way, so that all of the cultures shall experience the same variations in the surroundings. The cultures of a series are usually exposed by being placed side by side in some definite arrangement, upon a table or bench, the arrangement being sometimes changed from time to time. The aerial conditions affecting the plants may vary widely, however, within the area occupied by such an arrangement of cultures, and they do not all experience the same environmental changes. For example, cultures are almost certain to shade each other, some thus receiving more light than others, and cultures entirely surrounded by other cultures are undoubtedly subjected to a lower degree of atmospheric evaporating power than those not so surrounded. These undesirable inequalities of treatment were avoided in the present work by placing all the cultures upon two rotating tables, which stood side by side in the greenhouse.

The rotating tables here employed are constructed in the following manner. A motor-cycle wheel is mounted in a horizontal position upon a heavy, iron tripod-base, especially constructed for the purpose and having a radius of 53 cm. The wheel is provided with a special axle, extended at one end beyond the cone, to form a projecting piece 5 cm. in length and 2.2 cm. in diameter. This projection is tightly fitted into a suitable opening in the center of the tripod. Upon the wheel thus mounted, rests a circular wooden platform 1.2 meters in diameter, which is firmly secured by screws through the rim of the wheel. The platform itself is three-ply, with the grain of the wood crossed, and it is further prevented from warping by radial cleats bolted to its lower surface, and by its attachment to the rim. Such tables are easily capable of supporting a weight of 150 pounds, distributed uniformly about the center.

The tables were rotated by a small electric motor, belted to a reducing gear, which, in turn, was belted to the table. The belt connecting the first table to the reducing gear and also that joining the two tables were applied directly to the rims of the motor-cycle wheels. Each table made one complete revolution in about four minutes, thus exposing all the cultures to approximately similar changes of light, heat, and moisture conditions. The tables containing the cultures were rotated continuously during the entire time of an experiment, excepting that they were stopped for a short period every three days to allow the solutions to be changed.

The three series of wheat cultures here considered were conducted simultaneously for a period of twenty-three days after the plants were placed in the nutrient solutions. The nutrient solutions were renewed every third day. Each cork stopper, with its plants, was removed from its bottle and was transferred, as quickly and as carefully as possible, to another bottle containing the new solution. The used culture solution was discarded, after its volume had been determined. The original amount of solution (250 cc.) minus the amount remaining in a culture bottle when the solution was changed was, of course, a measure of the absorption (and approximately of the transpirational loss) for the culture in question and for the given time period. Data of the total transpirational water loss for each culture for the entire growth period, as well as for the partial periods between each two changes of solutions, were thus obtained. The used culture bottles were thoroughly cleaned, being rinsed first with tap water and then with distilled water, before each new filling.

During the period of growth, the general character of the development of tops and roots, as well as any unusual phenomena occurring in the plants, were noted. At the end of an experiment, the tops of the plants were severed from the roots just above the remains of the seed. The tops and roots were then dried separately in an electric oven at a temperature of from 75° to 80°C., for a period of about twenty-four hours, after which they were dried to constant weight at a temperature of from 102° to 104°C. The weighing bottles were transferred from the oven to a desiccator and were allowed to cool before weighing.

Records of temperature and of the evaporating power of the air in the greenhouse room where the cultures were carried out, were kept throughout the experiments. Temperature changes were recorded by means of a thermograph protected from direct sunlight and standing beside the rotating tables. The evaporating power of the air was measured by means of standardized porous cup atmometers;[17] several of these instruments were placed among the cultures on the rotating tables and daily readings were taken. Readings were corrected by multiplying by the coefficient of correction of the cup used.[18] The cups were frequently restandardized, but no marked alteration in the coefficients was detected, although the cups were not cleaned throughout the entire period of operation.

The first series of cultures was conducted in September, 1914, and this was repeated in November.

RESULTS AND SPECIAL DISCUSSION

1. INTRODUCTORY

Each of the two triple series of cultures here considered extended over a time period of twenty-three days. Series A, sub-optimal, optimal, and supra-optimal concentrations, was carried out between September 8 and October 1, 1914. Series B was exactly like series A but was carried out between November 13 and December 6. During the period of series A, the maximum temperature experienced by the cultures was 32°C. (on September 23) and the minimum was 12°C. (on September 29). The water loss from the porous cup atmometer, indicating the evaporating power of the air, gave a daily mean of 7.7 cc., a maximum daily rate of 14.3 cc. (on September 16), a minimum daily rate of 3.4 cc. (on September 12), and a total loss from the instrument of 177 cc. for the entire time period. During the growth period of series B a maximum temperature of 28°C. occurred on November 23, and a minimum of 13°C. was reached on November 18. The evaporation rate from the atmometer gave a daily mean of 10.8 cc., a maximum daily rate of 14.2 cc. (on December 4), a minimum daily rate of 10.4 cc. (on November 30), and a total loss from the instrument of 248 cc. for the entire time period. These atmometer readings are all corrected to the Livingston cylindrical standard.

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[17] Livingston, B. E., Atmometry and the porous cup atmometer. Plant World **18:** 21–30, 51–74, 95–111, 143–149. 1915. Also reprinted, Tucson, 1915. (This paper gives numerous references to the earlier literature of the instrument.)

[18] The cups used were obtained from the Plant World, Tucson, Arizona.

The present section will deal with the growth of the plants in the various cultures, as showing the physiological effects produced by the different total concentrations and the different salt proportions. The responses of the plants to the solution in which they grew will here be studied by means of three kinds of plant measurements, two of them strictly quantitative and the other more qualitative. The most useful quantitative measurements are in terms of the dry weights obtained from the cultures at the end of the period of growth. The more qualitative comparisons are made in terms of the apparent condition of the plants at the end of the culture period, and they deal mainly with unusual or pathological conditions as these might be detected by general observation. Finally, the second quantitative method of comparing the plants of the various cultures is by means of the relative amounts of water lost by transpiration during the entire culture period. These transpiration values will be considered in connection with the corresponding dry weights, to give the water requirements of the different cultures.

The results obtained by these three methods of study will be set forth in order.

2. DRY WEIGHTS

A. Presentation of dry weight data for tops and roots

Since the tops and roots were weighed separately, there are two sets of dry weight measurements available for each culture, both of which are of great interest. Also, by summing these two weights the total dry weight of any culture (six plants) may be obtained. Since, however, these sums are so markedly dominated by the dry weights of tops, they are of comparatively little interest here, and need not be further considered.

The numerical data of the yields of tops and of roots are presented in tables III, IV, and V. Each of these tables gives the dry weights of tops and of roots for series A and B, for a single one of the three total concentrations here employed. The first column gives the culture numbers, corresponding to their positions on the triangular diagram showing the variations in salt proportions (fig. 1). Then follow three columns referring to tops and three referring to roots. The first two columns in each of these groups of three present the data for series A and B, respectively, and the third column in each group gives the numbers obtained by averaging the corresponding data from series A and B. Each of these averages thus represents the two series combined. Each of the data is expressed in terms of the corresponding datum

TABLE III

Relative dry weights of tops and roots of wheat grown 23 days in three-salt solutions having sub-optimal total concentration (0.1 atm.); series A conducted from September 8 to October 1, series B, from November 13 to December 6, 1914.

CULTURE NUMBER	TOPS (6 PLANTS)			ROOTS (6 PLANTS)		
	Series A	Series B	Average	Series A	Series B	Average
R1C1	1.00* (0.2454)	1.00 (0.2748)	1.00L (0.2601)	1.00* (0.0948)	1.00 (0.1088)	1.00 (0.1036)
C2	1.32	1.11	1.22	1.13	0.97	1.05
C3	1.30	1.20	1.25	1.11	0.87	0.99
C4	1.25	1.34	1.30	0.87	0.95	0.91
C5	1.37	1.10	1.24	1.01	0.70	0.86L
C6	1.42	1.33	1.38	1.09	0.87	0.98L
C7	1.27	1.22	1.25	0.87	0.69	0.78L
C8	1.38	1.08	1.23	0.94	0.66	0.80L
R2C1	1.09	0.97	1.03L	1.27	1.11	1.19
C2	1.24	1.16	1.20	1.05	1.03	1.04
C3	1.47	1.31	1.39	1.00	0.85	0.93
C4	1.58	1.38	1.48	1.05	0.82	0.94
C5	1.38	1.42	1.40	0.80	0.73	0.77L
C6	1.40	1.46	1.43	0.78	0.73	0.76L
C7	1.47	1.30	1.39	0.97	0.62	0.80L
R3C1	1.27	0.94	1.11L	1.60	1.17	1.39H
C2	1.34	1.22	1.28	1.23	1.14	1.19
C3	1.47	1.37	1.42	0.96	1.04	1.00
C4	1.63	1.51	1.57H	0.95	0.92	0.94
C5	1.54	1.49	1.52H	0.93	0.70	0.82L
C6	1.60	1.10	1.35	0.97	0.85	0.91
R4C1	1.04	0.95	1.00L	1.45	1.17	1.31H
C2	1.33	1.09	1.21	1.35	1.09	1.22H
C3	1.49	1.33	1.41	1.23	0.88	1.06
C4	1.52	1.62	1.57H	0.82	0.95	0.89L
C5	1.66	1.63	1.65H	1.01	0.76	0.89L
R5C1	1.19	0.99	1.09L	1.36	1.23	1.30H
C2	1.35	1.58	1.47	1.25	1.11	1.18
C3	1.50	1.31	1.41	1.04	0.76	0.90
C4	1.32	1.31	1.32	0.75	0.68	0.72L
R6C1	1.25	0.95	1.10L	1.48	1.13	1.31H
C2	1.45	1.13	1.29	1.07	1.07	1.07
C3	1.52	1.36	1.44	1.19	0.85	1.02
R7C1	1.26	0.96	1.11L	1.52	1.19	1.36H
C2	1.38	1.20	1.29	1.40	1.16	1.28H
R8C1	1.24	0.96	1.10L	1.53	1.17	1.35H
K**	1.24	1.46	1.35	0.98	0.78	0.88
T**	1.62	1.61	1.62	0.92	0.91	0.92

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* The dry weight of culture R1C1 is always taken as unity and the other weights are expressed in terms of this. The actual dry weight of culture R1C1 is given in parenthesis, in grams.

** K and T represent Knop's solution and Tottingham's best solution for wheat tops, respectively. These data are introduced for comparison.

TABLE IV

Relative dry weights of tops and roots of wheat grown 23 days in three salt solutions having optimal total concentration (1.75 atm.); series A conducted from September 8 to October 1, series B, from November 13 to December 6, 1914.

CULTURE NUMBER	TOPS (6 PLANTS)			ROOTS (6 PLANTS)		
	Series A	Series B	Average	Series A	Series B	Average
R1C1	1.00* (0.4038)	1.00 (0.4170)	1.00L (0.4104)	1.00* (0.1016)	1.00 (0.1099)	1.00 (0.1058)
C2	1.26	1.12	1.19	1.26	0.95	1.11H
C3	1.10	1.29	1.20	0.90	0.96	0.93L
C4	1.21	1.12	1.17	1.11	1.02	1.07H
C5	1.24	1.27	1.26	1.02	0.95	0.99
C6	1.27	1.05	1.16	1.10	0.96	1.03
C7	1.17	1.04	1.11	1.04	0.98	1.01
C8	1.23	1.10	1.17	1.02	0.88	0.95
R2C1	1.02	1.03	1.03L	0.97	0.94	0.96
C2	1.23	1.05	1.14	1.16	0.94	1.05H
C3	1.28	1.22	1.25	1.11	0.75	0.93L
C4	1.34	1.19	1.27	1.07	0.83	0.95
C5	1.15	1.20	1.18	0.95	0.85	0.90L
C6	1.26	1.19	1.22	1.00	0.96	0.98
C7	1.33	1.13	1.23	1.17	0.90	1.04
R3C1	1.18	1.11	1.15	1.07	0.96	1.02
C2	1.29	1.19	1.24	1.21	0.93	1.07H
C3	1.38	1.34	1.36H	1.18	0.96	1.07H
C4	1.27	1.28	1.28	0.95	0.94	0.95
C5	1.23	1.27	1.25	0.93	0.90	0.92L
C6	1.23	1.31	1.27	0.94	0.91	0.93L
R4C1	1.13	1.11	1.12	1.02	1.05	1.04
C2	1.36	1.19	1.28	1.15	1.04	1.10H
C3	1.32	1.20	1.26	0.96	0.85	0.91L
C4	1.33	1.21	1.27	0.97	0.84	0.91L
C5	1.38	1.21	1.30H	1.08	1.00	1.04
R5C1	1.18	1.20	1.19	1.07	1.06	1.07H
C2	1.41	1.36	1.39H	1.14	1.01	1.08H
C3	1.30	1.17	1.24	0.97	0.85	0.91L
C4	1.37	1.18	1.28	1.10	0.95	1.03
R6C1	1.21	1.13	1.17	1.14	0.98	1.06H
C2	1.27	1.10	1.19	0.97	0.85	0.91L
C3	1.30	1.12	1.21	0.93	0.81	0.87L
R7C1	1.22	1.09	1.16	1.10	0.95	1.03
C2	1.33	1.28	1.31H	1.04	0.91	0.98
R8C1	1.23	....	1.23	1.21	....	1.21
K**	1.16	1.02	1.09	1.01	0.88	0.95
T**	1.22	1.32	1.27	1.08	0.96	1.02

* The weight of culture R1C1 is always taken as unity and the other weights are expressed in terms of this. The actual dry weight of culture R1C1 is given in parenthesis, in grams.

** K. and T represent Knop's solution and Tottingham's best solution for wheat tops, respectively. These data are introduced for comparison.

TABLE V

Relative dry weights of tops and roots of wheat grown 23 days in three-salt solutions having supra-optimal total concentration (4.0 atm.); series A conducted from September 8 to October 1, series B, from November 13 to December 6, 1914.

CULTURE NUMBER	TOPS (6 PLANTS)			ROOTS (6 PLANTS)		
	Series A	Series B	Average	Series A	Series B	Average
R1C1	1.00*	1.00	1.00L	1.00*	1.00	1.00L
	(0.2668)	(0.3628)	(0.3598)	(0.0938)	(0.0798)	(0.0868)
C2	1.13	1.12	1.13	0.94	1.14	1.04L
C3	....	....	....	....	....	....
C4	....	....	....	....	....	....
C5	....	....	....	....	....	....
C6	....	....	....	....	....	....
C7	....	....	....	....	....	....
C8	0.97	1.29	1.13	0.78	1.45	1.12
R2C1	0.88	1.06	0.97L	0.99	1.21	1.10
C2	1.14	1.13	1.14	1.06	1.20	1.13
C3	1.33	1.25	1.29H	1.19	1.16	1.18
C4	....	....	....	....	....	....
C5	....	....	....	....	....	....
C6	1.27	1.33	1.30H	1.08	1.33	1.21
C7	....	....	....	....	....	....
R3C1	0.99	1.10	1.05	1.01	1.21	1.11
C2	1.03	1.16	1.10	1.08	1.24	1.16
C3	1.24	1.28	1.26H	1.09	1.27	1.18
C4	1.20	1.28	1.24	1.01	1.19	1.10
C5	1.26	1.25	1.26H	1.11	1.25	1.18
C6	1.18	1.09	1.14	1.19	1.29	1.24
R4C1	1.02	1.11	1.07	1.16	1.40	1.28H
C2	1.10	1.36	1.23	1.12	1.39	1.26H
C3	1.34	1.42	1.38H	1.23	1.45	1.34H
C4	1.16	1.27	1.22	1.18	1.38	1.28H
C5	1.16	1.30	1.23	1.18	1.30	1.24
R5C1	0.91	1.05	0.98L	1.14	1.42	1.28H
C2	1.14	1.27	1.21	1.18	1.30	1.24
C3	1.15	1.29	1.22	1.05	1.25	1.15
C4	1.23	1.18	1.21	1.18	1.28	1.23
R6C1	0.98	1.12	1.05	1.17	1.40	1.29H
C2	1.14	1.20	1.17	1.19	1.24	1.22
C3	1.01	1.15	1.08	1.08	1.18	1.13
R7C1	1.00	1.02	1.01L	1.22	1.25	1.24
C2	1.02	1.06	1.04	1.19	1.24	1.22
R8C1	0.94	0.86	0.90L	1.22	1.14	1.18
K**	1.09	1.00	1.05	0.97	1.02	1.00
T**	1.23	1.22	1.23	1.24	1.25	1.25

* The dry weight of culture R1C1 is always taken as unity and the other weights are expressed in terms of this. The actual dry weight of culture R1C1 is given in parenthesis, in grams.

** K and T represent Knop's solution and Tottingham's best solution, respectively. These data are introduced for comparison.

for culture R1C1, considered as unity, but the actual dry weight for this culture is given in parentheses, in grams. The actual weight, in any case, may be obtained by multiplying the relative weight by the actual weight of culture R1C1 as given in the same column. The maximum yield in each series is here indicated by black-face type. The last two items in each column give the data obtained from cultures in Knop's solution and in Tottingham's best solution for wheat tops, with total concentrations equal to that of the three-salt solution considered in the same table. As has been remarked the supra-optimal series was not complete, on account of the instability of certain solutions. Also, culture R8C1 of optimal series B suffered an early death, for some reason not related to the properties of this particular solution; its data are therefore omitted.

In the discussion of these data the dry yields of tops will be treated first, followed by a similar treatment of root yields. For facility in making comparisons, the dry weights of tops and of roots relative to that of culture R1C1 for each actual series and for the three series of averages were plotted on triangular diagrams corresponding to that shown in figure 1. Only the three diagrams for average yields will be presented here, however (figs. 2-4 and 6-8), and only these averages will be discussed in detail. While considerable difference occurred between the two series (A and B) of each pair, these differences must be related to unknown conditions, and the most prominent features of both individual series of each pair are clearly brought out by the corresponding series of averages. (The diagrams of these figures also show the ion ratio values for the different culture solutions, which will be considered below. For the present discussion the narrow lines crossing the triangles may be ignored altogether, as well as the marginal numbers designating these lines, and also the ratio formulas.)

At the intersections showing the culture locations on the diagrams of figures 2-4 and 6-8, were placed numbers representing the relative average dry weights, taken directly from the proper columns of tables III, IV, and V. Thus each diagram represented graphically the distribution of average dry-weight magnitudes or yields in its particular series. To facilitate the study of this distribution, the total range of yields was divided into a lower one-fourth, a medium one-half, and an upper one-fourth, and three regions were outlined on each triangle, corresponding to these three partial ranges. (In tables III, IV, and V, the average yields are marked with an L if they lie within the lower one-fourth of the total range for the series, and with an H if they lie within the upper one-fourth.) The low and the high regions were then separated from the medium region by broken lines, each drawn through points showing equal values. These lines are shown in figures 2-4 and 6-8, but the yield values are omitted from the diagrams, to avoid complication. Also, the culture numbers are omitted from these triangles; they are of course the same as those shown in figure 1. One of the broken lines thus drawn upon the triangular diagram represents the upper limit of the area of low average dry weight (thus separating the lower one-fourth from the medium one-half) and the other indicates the lower limit of the area of high average weights (separating the upper one-fourth from the medium one-half). High areas (very good growth) are indicated by crosses and low areas (very poor growth) by small circles. The position on each diagram of the culture giving the greatest average dry weight is shown by a larger circle.

B. Dry weights of tops

Inspection of the columns referring to tops in tables III, IV and V, and of the triangular diagrams of figures 2–4, brings out the fact that the yield of tops is directly related to the total osmotic concentration and to the osmotic proportions of the salts in the culture solutions, as was of course to be expected. The following paragraphs will deal with these relations. The points to be brought out will be presented in three groups, referring to the three total concentrations employed. The discussion will proceed with reference to the triangular diagrams of figures 2–4, and will deal especially with the ranges of the high and low average dry weights, or the extents of the corresponding high and low areas as marked on the diagrams. In this study it is to be remembered that the position of any culture on the triangular diagram, and the range of any area, is a graphic representation of the osmotic proportions

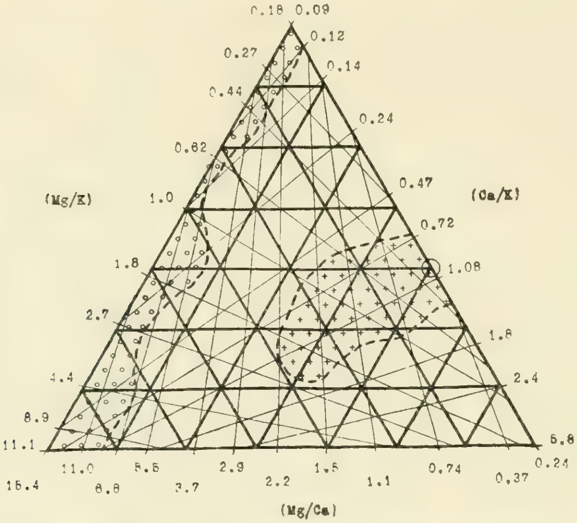

FIG. 2. Diagram showing relative yields of wheat tops in *sub-optimal* total concentration (0.1 atm.), averages from series A and B. Area of low yields (1.00 to 1.16) indicated by small circles; area of high yields (1.49 to 1.65) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

of the three salts as they occur in that culture solution, or of the range of these proportions in the cultures giving high or low average dry weights of tops. To interpret these positions upon the diagrams in terms of osmotic proportions of salts reference is to be had to figure 1.

(1) *Sub-optimal total concentration (0.1 atm.) (fig. 2).* The average dry weights from sub-optimal series A and B (plotted on the diagram of figure 2) indicate an area of low average weights (1.00–1.16) including the entire row of cultures on the left margin of the triangle, while the area of high average weights (1.49–1.65) occupies the right central region.

The highest average dry yield of tops occurs with culture R4C5 and is 65 per cent higher than the corresponding average for culture R1C1. The same culture gave the highest actual yield in both series A and series B, the increase over that of culture R1C1 being 66 per cent for the first and 63 per cent for the second series. From this agreement it appears that the greatest production of dry weight of tops in this three-salt solution with 0.1 atmosphere of diffusion tension is to be expected with the salt proportions of culture R4C5. This solution is characterized by having five-tenths of its total diffusion tension due to calcium nitrate, one-tenth due to magnesium sulphate, and four-tenths due to mono-potassium phosphate.

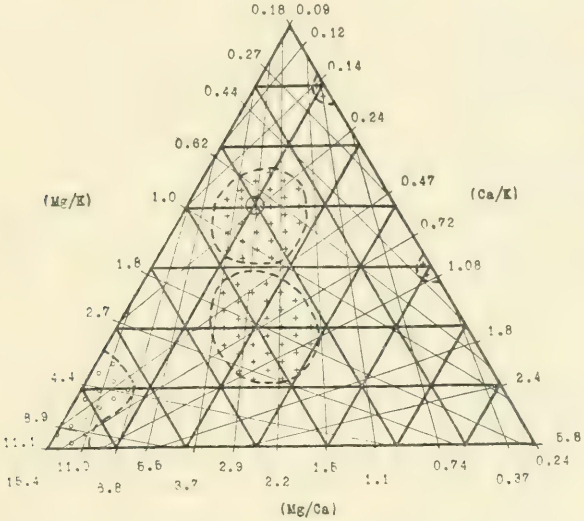

FIG. 3. Diagram showing relative yields of wheat tops in *optimal* total concentration (1.75 atm.), averages from series A and B. Area of low yields (1.00 to 1.10) indicated by small circles; area of high yields (1.29 to 1.39) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

(2) *Optimal total concentration (1.75 atm.) (fig. 3).* The diagram representing the average yields for the optimal total concentration shows an area

of low yields (1.00 1.10) at the extreme lower left. It shows two main areas of high yields (1.29 1.39) in the left central region, which are nearly joined at cultures R4C2 and R4C3. Secondary areas of high yield are also indicated for cultures R4C5 and R7C2, but these yields are not relatively very high.

Optimal series A and B agree in showing the highest dry weight of tops for the same culture, R5C2. In series A the yield for this culture was 41 per cent, and in series B 36 per cent higher than the corresponding dry yield for culture R1C1. The highest average yield of tops, of course, also occurs for culture R5C2, and is 39 per cent higher than the average yield for culture R1C1. It thus appears that the greatest production of dry weight of tops in this three-salt solution with 1.75 atmospheres of total diffusion tension is to be expected with the salt proportions of solution R5C2. This solution is characterized by having two-tenths of its total diffusion tension due to calcium nitrate, three-tenths due to magnesium sulphate, and five-tenths due to mono-potassium phosphate.

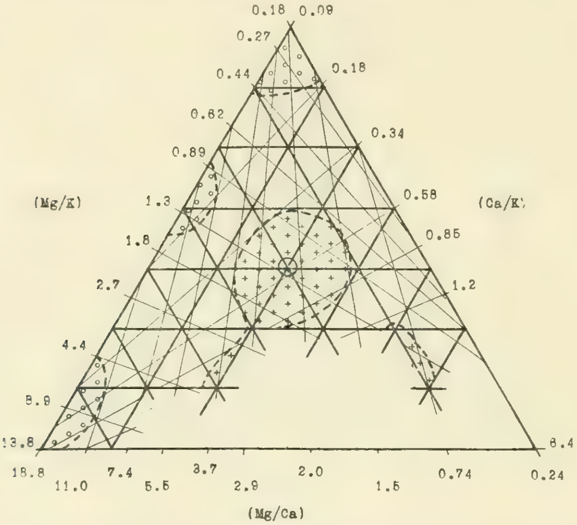

FIG. 4. Diagram showing relative yields of wheat tops in *supra-optimal* total concentration (4.0 atm.), averages from series A and B. Area of low yields (0.90 to 1.02) indicated by small circles; area of high yields (1.26 to 1.38) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

(3) *Supra-optimal total concentration (4.0 atm.) (fig. 4).* The incompleteness of the supra-optimal series, on account of the instability of some of the solutions, renders the location of the high and low areas on the diagram of figure 4 somewhat unsatisfactory. It appears highly probable that the main regions of high and low dry weights of tops are here rightly indicated, however.

The area of low average yields of tops (0.90–1.02) occupies three regions at the left margin of the diagram (fig. 4), and the main area of high average yields (1.26–1.38) occupies the center of the triangle.

The highest dry weight of supra-optimal series A occurred with culture R4C3 and was 34 per cent higher than that given by culture R1C1 of the same series. Similarly, the highest dry weight of series B occurred with the same culture and was 42 per cent higher than the corresponding weight of culture R1C1. The highest average yield (culture R4C3) is 38 per cent higher than that for culture R1C1. It thus appears that the greatest production of dry weight of tops in this three-salt solution with 4.0 atmospheres of total diffusion tension is to be expected with the salt proportions of culture R4C3. This solution is characterized by having three-tenths of its total diffusion tension due to calcium nitrate, three-tenths due to magnesium sulphate, and four-tenths due to mono-potassium phosphate.

(4) *Relation of total concentration to the physiological effects of the various osmotic proportions of the salts. Consideration of relative dry weights of tops (figs. 2–4).* It is important to appreciate the effect of the total concentration of the medium in determining the relative physiological values of the various salt proportions represented in each series. This may be done by referring to the three triangular diagrams representing average dry weights of tops (figs. 2–4). The positions and ranges, on these diagrams, of the areas of low and high average dry weights, and the positions of the solutions showing maximum yields, as these positions and ranges are altered relative to the total concentration, will now receive attention.

In passing from the sub-optimal to the optimal series (from fig. 2 to fig. 3), it is seen at once that the extent of the area of low top yields becomes greatly reduced. While this area extends along the entire left margin of the diagram representing the sub-optimal series (fig. 2), it is restricted to a small region at the lower left in the diagram representing the optimal (fig. 3). While eight solutions lie within this area on the sub-optimal diagram, only two (R1C1 and R2C1) are thus included on the other. Nevertheless, it is to be noted that these two solutions, the dry weights for which lie within the lower one-fourth of the total range of these weights for the optimal series, are the same solutions (characterized by the same salt proportions) that show the lowest two dry weights in the sub-optimal series. It is to be noted further, that the remaining left-marginal solutions of the optimal diagram (fig. 3) are all characterized as giving very low medium weights. These two total concentrations thus agree in indicating solutions R1C1 and R2C1 as very poorly adapted to the production of dry weights of tops of wheat as here grown. They also agree in showing the entire left marginal row of solutions on the triangular diagram as physiologically poor.

Turning to the supra-optimal diagram (fig. 4), the area of low top yields appears here in three separate portions lying at the left margin of the triangle, and the marginal solutions that lie between these separate portions of the area of low yields show very low medium weights. Thus the solutions of the left marginal row are indicated as of poor physiological balance for all three concentrations. In the supra-optimal series, solutions R1C1 and R2C1 (which show the two lowest yields in both of the other two series, as has been noted) do not show the lowest yields, but the next to the lowest is here given for one of these two, R2C1 (0.97).

It is apparent that some differences are manifest between the physiological properties of the left marginal solutions, according to the total concentrations, but these differences are not greatly marked. These eight solutions are characterized by low osmotic partial concentrations of calcium nitrate, but they include the whole range of partial concentrations in the case of both of the other salts. The worst two solutions (R1C1 and R2C1) are characterized by low partial concentrations of mono-potassium phosphate and high partial concentrations of magnesium sulphate. It thus appears that physiological balance is greatly disturbed by low partial concentration of the calcium salt, and this disturbance tends to be aggravated by relatively high partial concentrations of magnesium sulphate accompanied by low ones of mono-potassium phosphate.

Turning now to the areas of high relative dry weights, as represented on the diagrams of figures 2–4, if we pass from the sub-optimal to the optimal triangle (from fig. 2 to fig. 3), it appears as though the high area of the former drew away from the right margin and became more centralized, also dividing into two separate portions. These two portions of the high area of the optimal diagram (fig. 3) lie mainly to the left of the central vertical axis of the triangle and do not extend to the margin at any point. Passing to the supra-optimal diagram (fig. 4), these two regions of high yields appear to become combined in a more restricted region about the center of the diagram, but the supra-optimal series is not perfectly satisfactory, on account of incompleteness due to the chemistry of the salts.

If we compare the three solutions that show highest average yields of tops in the three series, respectively, we find that the best proportions of salts are not at all the same for the three different total concentrations here tested. With the sub-optimal total concentration (fig. 2) the best physiological balance is shown for solution R4C5, which is characterized by having only one-tenth of its total diffusion tension due to $MgSO_4$ while the remaining nine-tenths of the total diffusion tension is almost equally divided between the other two salts, five-tenths being due to $Ca(NO_3)_2$ and four-tenths to KH_2PO_4. With the optimal total concentration (fig. 3), solution R5C2 shows the best yield, and this solution occupies an entirely different position on the diagram from that occupied by R4C5. This solution has a relatively low partial diffusion tension due to $Ca(NO_3)_2$ (two-tenths), and a relatively high osmotic content of $MgSO_4$ (three-tenths). With the supra-optimal total concentration (fig. 4) the solution showing the highest dry weight of tops occupies still another position on the diagram. This solution has equal osmotic proportions of $Ca(NO_3)_2$ and $MgSO_4$, three-tenths of its total diffusion tension being due to each of these salts, and four-tenths are due to KH_2PO_4.

From these considerations it may be safely concluded, as has already been pointed out by Gile,[19] McCool,[20] and Tottingham, that physiological salt-balance in such nutrient media as these is markedly dependent upon the total concentration of the solution. It is therefore quite out of the question to state any given set of proportions of the three salts here used, that may be expected to give any particular kind of growth (as optimum, good, poor, etc.), unless the total concentration of the solution to be employed is also specified.

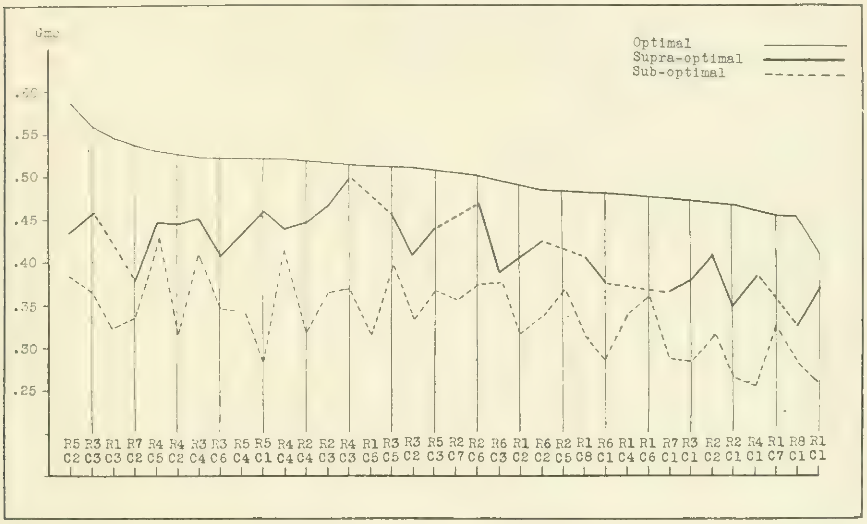

FIG. 5. Average actual yields (grams) of wheat tops (series A and B) for sub-optimal, optimal, and supra-optimal total concentrations.

Consideration of absolute dry weights of tops. The effect of the total concentration of the medium upon the influence of various salt proportions, as these determine physiological properties, may be brought out in another way, by considering the actual dry weights instead of the relative ones treated above. To bring out the relations in question the average yields of tops for the optimum concentration were arranged in the order of their magnitudes, beginning with the highest. These form a rather uniformly decreasing series of numbers, which were next plotted to form the graph shown as the upper one in figure 5. Here the abscissas are taken arbitrarily to represent the different cultures, the numbers of which are placed below, and the ordinates represent the average dry weight values. With the same abscissas, the corresponding dry weight values for the sub-optimal and supra-optimal concentrations were then plotted on the same sheet, using the same scale for the ordinates. As is shown in figure 5, there result three graphs, which lie one above the other. The upper one, representing the actual average yields of the optimal series, exhibits a uniform downward slope to the right, as was previously arranged. Next below this comes the supra-optimal graph, and that for the sub-optimal series is lowest.

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[19] Gile, P. L., Lime-magnesia ratio as influenced by concentration. Porto Rico Agric. Exp. Sta. Bull. 12. 1912.

[20] McCool, M. H., The action of certain nutrient and non-nutrient bases on plant growth. Cornell Agric. Exp. Sta. Mem. 2: 121-170.

From figure 5 it is at once clear that the supra-optimal and sub-optimal graphs, while showing a general downward slope to the right, are very irregular and not at all parallel to each other or to the optimal graph. This indicates again that the same set of salt proportions (denoted by the same culture number) has very different physiological properties with the three different total concentrations.

Furthermore, the optimal series definitely exhibits the greatest average absolute dry weight for every set of salt proportions throughout the entire series. There is no crossing of the graphs; every set of salt proportions in the supra-optimal series gave a lower dry weight average than did the same proportions in the optimal, and every one in the sub-optimal gave a lower average weight than did the same set in the supra-optimal. The fact that each series has its own particular set of optimal proportions of the three salts is again apparent in this series of graphs. It may be added that the highest yield for the optimal concentration is not equalled by the highest one for the supra-optimal, and that the highest for the supra-optimal is not equalled by that for the sub-optimal.

Relation of yields of tops to the proportions of the chemical ions. a. Ion ratios. In connection with the problems of salt antagonism and the physiological balance of nutrient solutions, considerable attention has been given by various writers to the ratios of partial salt (ion, element, etc.) concentrations. Such ratios are frequently stated in such a way as to show the number of atoms of one element occurring in the solution, for each single atom of another element, as in the case of Loew's[21] ratio of magnesium to calcium. Of course these ratios, like the partial concentrations from which they are derived, may also be expressed directly in terms of weight, thus giving the number of grams of one element present in the solution for a single gram of another element. But this sort of expression is practically without either chemical or physiological meaning, and therefore the weight method will not be here employed. It is obvious that the cations here dealt with are atoms, while the corresponding anions are atomic groups. It therefore follows that ratios of atoms and also those of atomic groups may be employed to characterize a given nutrient solution. Since ions are to be considered as the chemically and metabolically active units in such solutions as those here dealt with, a detailed study of ionic ratios has been carried out, with the aim of finding out whether certain ones of these various ratio values may be important in determining the physiological properties of the various salt combinations. The present section will be devoted to such a study.

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[21] Loew, O. and May, D. W., The relation of lime and magnesia to plant growth. U. S. Dept. Agric. Bur. Plant Ind. Bull. 1. 1901.

In the solutions here dealt with there are three cations and three anions, neglecting the very small amount of iron which is uniformly present. It follows that there are three possible cation ratios, Mg/Ca, Mg/K, and Ca/K, and their reciprocals, the latter not requiring special attention; also, there are three anion ratios, SO_4/NO_3, SO_4/PO_4, and NO_3/PO_4, and their reciprocals. This consideration neglects the H-ions that arise from KH_2PO_4, and assumes complete ionization of all the salts. From the nature of the salts used it is clear that the ratio SO_4/NO_3 always has a value just one-half as great as that of the ratio Mg/Ca for the same solution. Similarly, the ratio NO_3/PO_4 has twice the magnitude of the ratio Ca/PO_4, and the ratio SO_4/PO_4 is identical in value with the corresponding ratio Mg/K in the same solution. These relations follow from the fact that for every ion of Ca there are two ions of NO_3, while the ions of Mg and K are accompanied by single ions of SO_4 and PO_4, respectively. From the way in which these three-salt nutrient solutions are prepared, if the values of two out of the three cation ratios are known the value of the third may be found, since the total salt concentration of the solution is also known and only three salts ever enter into its composition. A similar relation holds for the magnitudes of the three anion ratios, and since these are definitely related to the cation ratio values, it follows that if the values of any two cation ratios or of any two anion ratios be given (of course not including both of any pair of reciprocals, which are really the same ratio), all the other ionic ratios may be directly calculated, including those formed from one cation and one anion.

The chemical nature of each of the solutions used in this study being thus determined by any two ion ratio values, taken together with the total concentration, it becomes of considerable importance to determine the ratios for all of the different solutions of each of the three series and to study these values with reference to the physiological properties of the various solutions.

On account of the known relations holding between the different ratio values, as noted above, it becomes unnecessary to determine any but the three different cation ratios for each solution. These determinations are accomplished by calculation directly from the partial molecular salt concentrations given in table II. Thus, in the case of solution R1C1 with optimal total concentration, for example, the partial molecular concentration of $MgSO_4$ is 0.0400, that of $Ca(NO_3)_2$ is 0.0026, and that of KH_2PO_4 is 0.0036, from which is appears that this solution contains, per liter, 0.0400 gram-ions (or gram-atoms) of Mg; 0.0026 gram-ions of Ca, and 0.0036 gram-ions of K. The three cation ratio values characterizing this solution are then obtained by performing the operations indicated by the ratios themselves: $\frac{Mg}{Ca} = \frac{0.0400}{0.0026} = 15.40$; $\frac{Mg}{K} = \frac{0.0400}{0.0036} = 11.10$, etc. These operations are of course justified by the chemical principle (based on the atomic theory of matter) that the number of atoms of an element present in a given mass of it is proportional to the number of gram-atoms contained in the mass, a gram-atom (similar to a gram-molecule) being the number of grams of the element in question that is represented by its atomic weight.

b. Presentation of ion ratio values. The ratio values, Mg/Ca, Mg/K, and Ca/K, for all the thirty-six sets of salt proportions and for all three total concentrations here studied, are set forth in table VI. It will be noted that the ratio values are the same for the sub-optimal and optimal concentrations, but that the supra-optimal concentration has a series of its own. This is on account of the apparent depression of ionization occurring in the stronger solutions, as has been noted above. The first column of table VI gives the solution numbers, denoting the salt proportions on the basis of partial diffusion tensions. Columns 2, 3 and 4 present the three ratio values for the sub-optimal and optimal concentrations, and columns 5, 6 and 7 give those for the supra-optimal. The solutions giving the greatest yields of tops and of roots are designated in the table by letters, which refer to notes below to this effect.

The data of table VI were plotted upon the triangular diagrams, in a manner similar to that in which the dry weights were plotted, after which lines were drawn through approximately equal ratio values, thus dividing the triangular area into strips with reference to each ratio. The diagrams thus obtained have been represented by the narrow lines in figures 2–4, and will be similarly employed in figures 6–8 and in figures 10 and 11, as bases on which the other data are plotted. It will be noted, from the figures just mentioned, that the cation ratio diagram for the sub-optimal and that for the optimal total concentration (figs. 2, 3, 6, 7) are identical, while the corresponding diagram for the supra-optimal concentration (figs. 4 and 8) is somewhat different, corresponding to the fact that each cation ratio of table VI has a different value for the sub-optimal and optimal series from that given for the supra-optimal series. As has been remarked, this is due to the depressed ionization in the more concentrated series of solutions. In these figures the narrow lines approximately represent the respective ratio values, while the broad lines show by their intersections the different osmotic salt proportions and the locations of the different cultures or solutions. Since there are three ratios to be considered, there are three sets of ratio lines shown on each triangle. Each side of the triangle is intersected by all of the lines

TABLE VI

Values of the three cation ratios for three-salt solutions with sub-optimal, optimal, and supra-optimal total osmotic concentration.

SOLUTION NUMBER	SUB-OPTIMAL AND OPTIMAL CONCENTRATION			SUPRA-OPTIMAL CONCENTRATION		
	Mg/Ca	Mg/K	Ca/K	Mg/Ca	Mg/K	Ca/K
R1C1	15.40	11.10	0.72	18.80	13.78	0.73
C2[a]	6.74	9.72	1.44	8.10	11.89	1.47
C3	3.85	8.34	2.16	....	....	....
C4	2.40	6.95	2.88	....	....	....
C5	1.54	5.55	3.60	....	....	....
C6	0.96	4.17	4.32	....	....	....
C7	0.55	2.78	5.04	....	....	....
C8	0.24	1.39	5.76	0.24	1.58	6.40
R2C1	13.46	4.86	0.36	16.21	5.99	0.37
C2	5.77	4.17	0.72	6.73	4.95	0.75
C3	3.21	3.47	1.08	3.60	3.95	1.10
C4	1.92	2.77	1.44	....	....	....
C5	1.15	2.08	1.80	....	....	....
C6	0.64	1.39	2.16	0.68	1.58	2.31
C7	0.27	0.69	2.52	....	....	....
R3C1[b]	11.55	2.78	0.24	13.46	3.30	0.24
C2	4.81	2.32	0.48	5.40	2.64	0.49
C3	2.56	1.85	0.72	2.88	2.11	0.73
C4	1.44	1.39	0.96	1.61	1.59	0.98
C5	0.77	0.93	1.20	0.86	1.05	1.22
C6	0.32	0.46	1.44	0.34	0.52	1.54
R4C1	9.61	1.74	0.18	10.89	1.94	0.18
C2	3.85	1.39	0.36	4.32	1.55	0.36
C3[c]	1.92	1.04	0.54	2.16	1.16	0.54
C4	0.96	0.69	0.72	1.07	0.77	0.72
C5[d]	0.38	0.35	0.90	0.43	0.39	0.90
R5C1	7.70	1.11	0.14	8.63	1.24	0.14
C2[e]	2.88	0.83	0.29	3.24	0.93	0.29
C3	1.28	0.56	0.43	1.44	0.62	0.43
C4	0.48	0.28	0.58	0.54	0.31	0.58
R6C1	5.77	0.69	0.12	6.49	0.74	0.11
C2	1.92	0.46	0.24	2.16	0.49	0.23
C3	0.64	0.23	0.36	0.72	0.24	0.34
R7C1	3.85	0.40	0.10	4.32	0.41	0.09
C2	0.96	0.20	0.20	1.07	0.20	0.19
R8C1	1.92	0.18	0.09	2.15	0.18	0.09

[a] Best solution for yield of roots, optimal concentration.

[b] Best solution for yield of roots, sub-optimal concentration.

[c] Best solution for yield of tops and roots, supra-optimal concentration.

[d] Best solution for yield of tops, sub-optimal concentration.

[e] Best solution for yield of tops, optimal concentration.

for just one ratio and by some of the lines for each of the other two ratios. The ratio values represented by the lines of each set are shown along that side of the triangle which is intersected by all of that particular set, and that side may be considered as the triangle base for the ratio in question. The three ratio formulas are placed just outside of the triangle, on the three sides that are their respective bases, Mg/Ca being below, Mg/K at the left, and Ca/K at the right. It will be noticed at once that the ratio values do not alter at a constant rate, proceeding across the triangular diagram, so that the scale represented by the spacing of the narrow lines is much larger at one end of each series of values than at the other. In this respect the ratio diagrams are fundamentally different from those of osmotic salt proportions, represented by the broad lines of the same figures.

c. Relation of ion ratio values to yields of tops. A study of the relations between the various ion ratio values and the magnitudes of the corresponding yields, shown in figures 2-4, brings out some interesting points. In the diagram for the sub-optimal series (fig. 2) the area of low top yields, extending along the left side of the triangle embraces the full range of the value of the ratio Mg/K, from 0.18 to 11.10. The magnitude of this ratio is therefore not at all related to the production of low top yields in this series. Each of the other two ion ratios, however, shows a definite relation to the position and extent of this low area, which embraces ratio values of Mg/Ca varying from 1.62 to 15.40, and of Ca/K varying from 0.09 to 1.21. The total range of each of the last two ratios is considerably greater than the range embraced by the low area of the diagram, so that there occurs a range of ratio values, in each case, that are characterized as *not* producing low top yields. It therefore follows that low top yields are to be expected, with this sort of 3-salt solution having a total concentration of 0.1 atmosphere, when the value of Mg/Ca lies between 1.62 and 15.40 while the value of Ca/K lies between 0.09 and 1.21. The cultures giving low yields of tops are characterized as including all but the very lowest values of Mg/Ca here tested and as restricted to the lowest values of Ca/K.

Similarly, for the area of high dry weights of tops, with the sub-optimal total concentration (fig. 2), this area is seen to be confined to ratio ranges as follows: Mg/Ca, 0.38–2.36; Mg/K, 0.31–2.58; and Ca/K, 0.65-1.43. These ranges are not very great in any case, and all lie near—but do not include—the lowest values of the respective ratios occurring in the series as here arranged. Since any two ratio values are sufficient to determine the position of a point on the triangular diagram, it may be said that high top yields may be expected in this series of solutions (0.1 atm. total concentration), when the value of Mg/Ca lies between 0.38 and 2.36, and that of Ca/K lies between 0.65 and 1.43. As the series is here arranged the similar range of the third ratio value (Mg/K) is also determined by these data.

The highest average yield of tops here occurred (culture R4C5) with the ratio values: Mg/Ca, 0.38; Mg/K, 0.35; and Ca/K, 1.08. This means that this best solution for tops contains 0.38 atom of Mg for 1 atom of Ca, 1.08 atom of Ca for 1 atom of K, and 0.35 atom of Mg for 1 atom of K. It thus contains the atoms Mg, Ca, and K in the proportions 0.35 : 1.08 : 1.00.

The remaining two diagrams of top yields (figs. 3 and 4) may be treated in a similar manner, thus bringing out the ranges of the various ratio values embraced by the low and high areas, respectively. The results of such comparisons are brought together in table VII. This table is divided into four parts. In the first part are given the minimum and maximum ratio values and the ranges for these, for the entire series. In the second part are given the ratio values and ranges for low yields of tops (lowest one-fourth of the total range of yields). In the third part are given the corresponding numbers for high yields (highest one-fourth of the total range of yields). In the fourth part are given, for ready comparison, the ratio values characterizing the solution that produced the highest yield for each of the three different total concentrations.

TABLE VII

Minimum and maximum values for the three cation ratios, and ranges of these values, for each entire series and for high and low yields of wheat tops, with each total concentration tested. Also, these ratio values for the three best solutions for yields of tops.

		Mg/Ca ratio			Mg/K ratio			Ca/K ratio		
		Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)	Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)	Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)
Entire series	Minimum	0.24	0.24	0.24	0.18	0.18	0.18	0.09	0.09	0.09
	Maximum	15.40	15.40	18.80	11.10	11.10	13.78	5.76	5.76	6.40
	Range	15.16	15.16	18.56	10.92	10.92	13.60	5.67	5.67	6.31
Low yields	Minimum	1.62	7.36	1.23	0.18	3.56	0.18	0.09	0.29	0.09
	Maximum	15.40	15.40	18.80	11.10	11.10	13.78	1.21	1.08	0.85
	Range	13.78	8.04	17.57	10.93	7.54	13.61	1.12	0.79	0.76
High yields	Minimum	0.38	1.58	0.74	0.31	0.53	0.74	0.65	0.19	0.35
	Maximum	2.36	5.16	4.07	2.58	2.84	4.45	1.43	1.45	2.31
	Range	1.98	3.58	3.33	2.27	2.31	3.71	0.78	1.26	1.96
Highest yields		0.38	2.88	2.16	0.35	0.83	1.16	1.08	0.29	0.54

The results shown in table VII are expressed graphically in figures 2-4, the first of which has been discussed. Reference to these figures and to table VII shows that the areas of low and high yields of tops are generally limited to certain ranges of the cation ratio values, these ranges being less extensive than the corresponding total ranges for the series in question.

There are just two exceptions to this last statement; as has been remarked, the magnitude of the ratio Mg/K is without correlation to the yield of tops in the sub-optimal series (fig. 2), and the same is true for the supra-optimal series (fig. 4). Also, the range of Mg/K that defines the area of low top yields in the optimal series (fig. 3) embraces somewhat more than the upper one-half of the total range of this value in this case, so that here also, this ratio does not appear to furnish a very definite criterion by which to judge the physiological properties of the medium. It is also to be noted that the ranges of the ratio Mg/Ca that correspond to the areas of low top yields in the sub-optimal (fig. 2) and supra-optimal (fig. 4) series are almost coextensive with the corresponding total ranges for the respective series and that the range, of this same ratio value, that limits the low yields in the optimal series (fig. 3), embraces almost the whole of the upper one-half of the corresponding total range. From these observations it appears that the solutions giving low dry weights of tops are not very satisfactorily defined in terms of either the Mg/Ca or the Mg/K ratio values, though these values offer somewhat better criteria for judging the physiological balance of the solution in the case of the optimal total concentration than in either of the others.

The areas representing low yields are well defined in all three diagrams (figs. 2–4) by the ranges of the ratio-value Ca/K, this range embracing only the lower one-fifth (or less) of the corresponding total range. With all three total concentrations the solutions giving high top yields are all clearly limited to low values of all three cation ratios, but in no case are any of these solutions characterized by the very lowest ratio values here considered. In general, no single ratio and no pair of ratios appear to determine the production of high top yields any more than that of low ones.

It is instructive to observe the marked differences between the atomic proportions of Mg, Ca, and K characterizing the solutions giving the highest yields of tops in the three series, respectively. While, as has been remarked, the most perfectly balanced solution (for dry weight of wheat tops) of the sub-optimal series (total concentration 0.1 atm.) contains 0.35 and 1.08 atoms of magnesium and of calcium, respectively, for each single atom of potassium, yet the most perfectly balanced solutions of the optimal and of the supra-optimal series (total concentrations 1.75 and 4.0 atm.) are very different in this respect. The greatest yield of tops was obtained in a solution having the atomic proportions, Mg, 0.83; Ca, 0.29; K, 1.00, with optimal total concentration, while the best solution for top growth with supra-optimal total concentration possessed the proportions, Mg, 1.16; Ca, 0.54; K, 1.00. It is thus obvious that the cation atomic proportions characterizing the best physiological balance, as here brought out, vary markedly, and in no simple manner, with the total concentration of the solution.

C. Dry weights of roots

Turning now to the dry weights of roots, as given in tables III, IV, and V, it appears that these, as well as the yields of tops, show marked variations according to the osmotic proportions and total osmotic concentrations of the solutions in which they grew. The following consideration of the average root yields will follow the same general lines as were followed in considering the average weights of tops, but will not be as detailed as was the former discussion. It will have reference to the triangular diagrams of figures 6–8.

(1) *Sub-optimal total concentration* (*0.1 atm.*) (*fig. 6*). The average dry weight of roots for the sub-optimal series ranges from 0.72 to 1.39, relative to the average yield for solution R1C1. The diagram for these root yields (fig. 6) shows an area of low relative values (0.72–0.89) occupying about a third of the entire triangle, about its lower right angle. The area of high root yields (1.22–1.39), on the other hand, occupies nearly the whole of the left marginal region and includes solutions R8C1 and R7C2 of the right margin. The greatest dry weight of roots for this total concentration is shown for solution R3C1, near the lower limit of the area of high root yields. This solution is characterized by having one-tenth of its total diffusion tension due to $Ca(NO_3)_2$, six-tenths due to $MgSO_4$, and three-tenths due to KH_2PO_4.

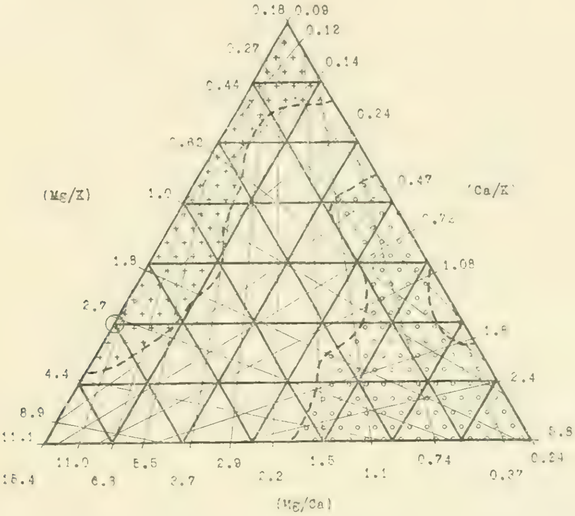

FIG. 6. Diagram showing relative yields of wheat roots in *sub-optimal* total concentration (0.1 atm.), averages from series A and B. Area of low yields (0.72 to 0.89) indicated by small circles; area of high yields (1.22 to 1.39) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

(2) *Optimal total concentration* (*1.75 atm.*) (*fig. 7*). The range of average relative root yields for the optimal series is from 0.87 to 1.11. Comparing

the diagram for this series (fig. 7) with that for the sub-optimal series (fig. 6) it appears as though the area of low relative yields had here migrated upward and to the left, while the area of high yields had correspondingly migrated downward and to the right. The main region of low dry weights of roots (0.87–0.93, fig. 7) lies mainly to the right of the central vertical axis of the triangle, but includes two solutions on that axis. The main region of high root yields (1.05–1.11) lies to the left of the central vertical axis, the greatest root yield being shown for solution R1C2, on the lower margin of the triangle. This solution is characterized by having two-tenths of its total diffusion tension due to $Ca(NO_3)_2$, seven-tenths due to $MgSO_4$, and one-tenth due to KH_2PO_4.

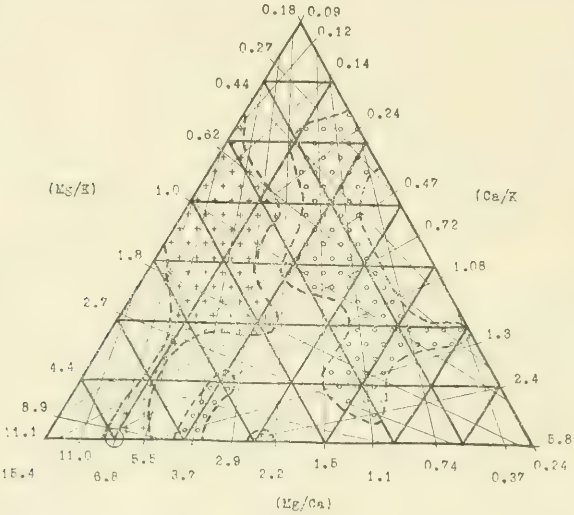

FIG. 7. Diagram showing relative yields of wheat roots in optimal total concentration (1.75 atm.), averages from series A and B. Area of low yields (0.87 to 0.93) indicated by small circles; area of high yields (1.05 to 1.11) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

(3) *Supra-optimal total concentration (4.0 atm.) (fig. 8).* The total range of average relative dry weights of roots for the supra-optimal series extends from 1.00 to 1.34. On the diagram for this series (fig. 8) the area of low average root yields (1.00–1.08) occupies the extreme lower left region of the triangle. The corresponding area of high average yields (1.26–1.34) here occupies the center of the triangle and is extended to the right, but not as far as the right margin, and also to the left, where it widens to include three solutions on the left margin. This triangle is somewhat unsatisfactory on account of its incompleteness (due to chemical relations, as has been stated), but there is no suggestion of an area of low yields at the right of the central axis (as in figs. 6 and 7), and it seems fair to suppose that the effect

of this high concentration has been to shift the region of poor growth as is here indicated. The highest average root yield is shown for solution R4C3, near the center of the diagram. This solution is characterized by having three-tenths of its total diffusion tension due to $Ca(NO_3)_2$, three-tenths due to $MgSO_4$, and four-tenths due to KH_2PO_4.

(4) *Relation of total concentration to the physiological effects of the various osmotic proportions of the salts. Consideration of relative dry weights of roots (figs. 6–8).* The effect of total concentration upon the positions and ranges of the areas of low and high average yields of roots, as shown on the triangular diagrams (figs. 6–8), will now receive attention.

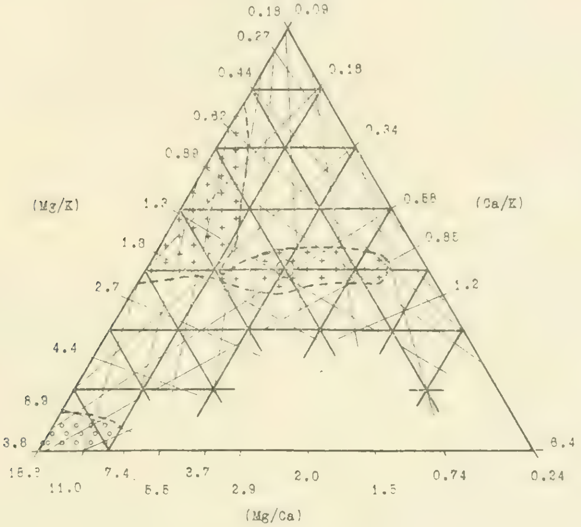

FIG. 8. Diagram showing relative yields of wheat roots in supra-optimal total concentration (4.0 atm.), averages from series A and B. Area of low yields (1.00 to 1.08) indicated by small circles, area of high yields (1.26 to 1.34) indicated by crosses. The culture giving the highest yield is marked by a larger circle.

Comparison of the diagrams of figures 6–8 makes it clear enough that total concentration has a very pronounced effect in determining the relative influence of the different salt proportions upon growth of roots as here measured. This effect is so pronounced that it is obviously impossible to select any single set of salt proportions which might agree, throughout the three total concentrations, in producing either a very low or a very high dry weight of roots. The study of the growth of roots in relation to their chemical and physical environment is in such an early stage, and the data here presented appear to be so complicated, that further generalization need not be undertaken. The point to be emphasized in this connection, as in the case of tops, is that a culture solution is not to be defined, as physiologically balanced for root growth, in terms of its salt proportions alone; its total concentration must also be stated.

Consideration of absolute dry weights of roots. The actual average dry weights of roots obtained in a period of twenty-three days, with the various concentrations and salt proportions here studied, will now be taken up, as was done for the corresponding average yields of tops. The average dry weights of roots for the optimum concentration were arranged in the order of their magnitudes, beginning with the highest, and, as in the case of tops, these numbers form a rather uniformly decreasing series, which is shown graphically as the uniformly sloping, nearly straight line of figure 9. This graph, and the remaining two graphs of that figure, are plotted in the same manner as was employed for tops (fig. 5). Unlike those for top yields, there is little or no tendency for these three graphs to show parallelism; frequent crossing is manifest. There is not even any marked tendency for the sub-optimal and supra-optimal graphs to slope downward to the right. Practically the only tendency toward any agreement is exhibited between the sub-optimal and supra-optimal graphs for the first nine cultures as arranged in figure 9. These two graphs appear, in this region, to rise and fall simultaneously, but the fifth culture (R1C4) of the supra-optimal series is omitted, so that even this amount of agreement is uncertain. It should be emphasized that the order in which the cultures are arranged for the graphs of figure 9, is not at all the same as their order in figure 5, so that these two sets of graphs

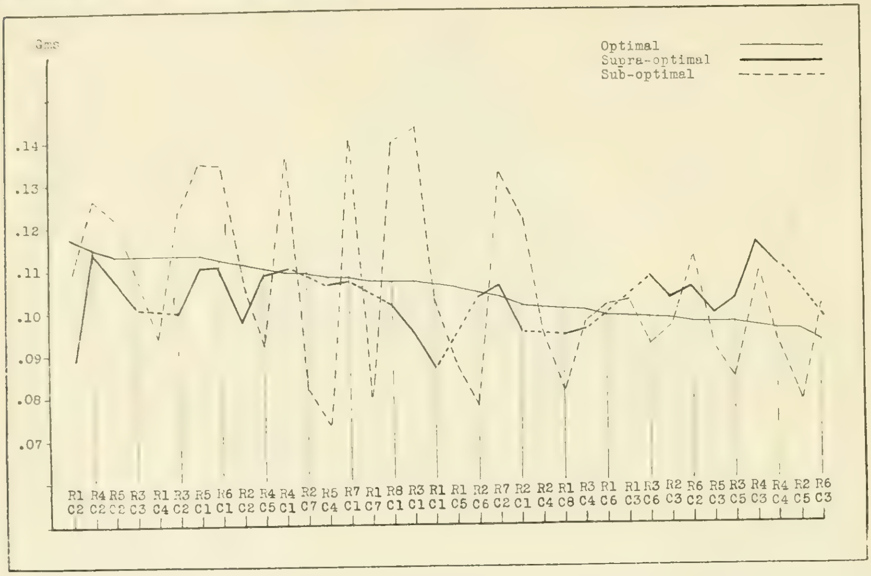

FIG. 9. Average actual yields (grams) of wheat roots for sub-optimal, optimal, and supra-optimal total concentrations.

are not directly comparable excepting in the general way just pointed out. (For a direct comparison see figure 15.)

It therefore appears, that the physiological value, for the production of root yields, of any given set of salt proportions unquestionably varies as the total concentration of the medium is altered, which is quite similar to the general conclusion reached from the study of top yields.

Relation of yields of roots to the proportions of the chemical ions. The discussion of root yields with reference to the proportions of the three cations in the solutions will follow the same general lines as were followed in the corresponding discussion of top yields, but it will be less detailed. The maximum and minimum ratio values for the regions of high and low root weights, as shown on the diagrams of figures 6, 7, and 8 are set forth in table VIII, which conforms in every way with table VII.

TABLE VIII

Minimum and maximum values for the three cation ratios, and ranges of these values, for each entire series and for high and low yields of wheat roots with each total concentration tested. Also, these ratio values for the three best solutions for yields of roots.

		Mg/Ca RATIO			Mg/K RATIO			Ca/K RATIO		
		Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)	Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)	Sub-optimal (0.1 atm.)	Optimal (1.75 atm.)	Supra-optimal (4.0 atm.)
Entire series	Minimum	0.24	0.24	0.24	0.18	0.18	0.18	0.09	0.09	0.09
	Maximum	15.40	15.40	18.80	11.10	11.10	13.78	5.76	5.76	6.40
	Range	15.16	15.16	18.56	10.92	10.92	13.60	5.67	5.67	6.31
Low yields	Minimum	0.24	0.32	7.52	0.25	0.21	8.18	0.46	0.22	0.44
	Maximum	2.06	2.21	18.80	6.37	3.56	13.78	5.76	3.59	1.46
	Range	1.82	1.89	11.28	6.12	3.35	5.60	5.30	3.37	1.02
High yields	Minimum	0.89	2.29	0.75	0.18	0.53	0.54	0.09	0.11	0.10
	Maximum	12.23	9.58	11.64	4.26	10.33	2.33	0.45	1.45	0.86
	Range	11.34	7.29	10.89	4.08	9.80	1.79	0.36	1.34	0.76
Highest yields		11.55	6.74	2.16	2.78	9.72	1.16	0.24	1.44	0.54

As in the case of the top yields, the areas of high and of low dry weights of roots are generally limited to certain ranges of the three cation ratio values here considered, these ranges being always less extensive than the corresponding total ranges. In two cases the limiting range of ratio values embraces almost all of the corresponding total range: (1) in the sub-optimal series the area of low root yields (fig. 6) embraces all but the very lowest values of the ratio Ca/K, and (2) in the optimal series the area of high yields (fig. 7) embraces all but the very lowest and very highest values of the ratio Mg/K.

For all three total concentrations, the solutions giving high root yields are clearly defined as having very low values of the Ca/K ratio and rather extensive medium ranges of the ratios Mg/Ca and Mg/K. Further details may be discovered from a study of table VIII and of the diagrams of figures 6–8, but their statement here would add nothing leading to valuable generalizations at present. It is clear that the ratio values characterizing solutions giving low or high yields vary in a pronounced way with the alteration of the total concentration.

The atomic proportions of Mg, Ca, and K that characterize the three solutions giving the highest yields of roots in each series deserve special attention here, as in the case of top yields. For the sub-optimal total concentration (0.1 atm.) the most perfectly balanced solution for root yields contains 2.78 atoms of magnesium and 0.24 atoms of calcium for each single atom of potassium. For the optimal series (total concentration 1.75 atm.), these atomic proportions are Mg, 9.72: Ca, 1.44: K, 1.00, and for the supra-optimal series (total concentration 4.0 atm.), they are Mg, 1.16: Ca, 0.54: K, 1.00. The last of these sets of proportions is the only one of the three that is the same for the highest yields of both roots and tops. The cation atomic proportions characterizing the best physiological balance for root yields, as here brought out, vary as markedly and in as recondite a way, with variation in total concentration, as they do in the case of yields of tops.

3. APPARENT CONDITION OF PLANTS

Condition of tops

No apparent differences (aside from those of size) in the tops of the wheat plants of any series occurred during the first ten days of their growth, and none appeared at all in the sub-optimal series. In the optimal and supra-optimal concentrations, however, evidences of disturbed growth appeared in the tops of a number of cultures, usually about the tenth day after the young plants had been placed in the solutions. This disturbance was practically identical with that described by Tottingham and called by him magnesium injury. It manifested itself in two forms.

The more pronounced of these forms of injury consisted in a lateral rolling backward of the entire length of the injured leaves. Leaves so injured showed the effect at a very early stage and never attained the mature form and size. The young leaves, just emerging from the sheath, rolled back laterally throughout their entire length, frequently also coiling into a close spiral. The first few leaves produced by the plants usually escaped injury altogether or suffered only from the less pronounced form to be described below, but the later leaves (in cultures where pronounced injury occurred) were attacked at a very early stage of their development, even while they were emerging from the sheath.

The pronounced form of injury did not occur on leaves that had already developed to the usual size, though such leaves were frequently affected by the less pronounced form. This form of injury manifested itself, in either young or old leaves that had previously appeared normal, by a yellowing and shrivelling of a small region several centimeters from the tip. The distal portion, above the first lesion, remained green and apparently in healthy condition for some time, but finally died. This less pronounced form of injury usually began with the third leaf formed, mainly after the leaf had attained a considerable size. It did not appear to retard growth perceptibly; indeed, the largest and most vigorous cultures, in both optimal and supra-optimal series, always showed this less pronounced form of leaf injury. Where both forms of injury occurred on the same plant, the less pronounced form was the first to make itself evident.

The cultures of the optimal and supra-optimal series may be divided, according to the injury they sustained, into three classes: (1) those that were without injury, (2) those that exhibited only the less pronounced form of injury, and (3) those that exhibited the more pronounced form. For the optimal total concentration of the solutions the observations on leaf injury, from both series A and B, are summarized in the triangular diagram of figure 10, and a similar presentation of these observations for the supra-optimal concentration of the solutions is given in the diagram of figure 11. In these diagrams the cultures suffering from the pronounced form of injury occupy the area denoted by crosses. These cultures were also affected by the less pronounced form of injury. Those exhibiting only the less pronounced form occupy the area denoted by small circles. The area representing uninjured cultures is unshaded. The three areas thus shown for each diagram are separated, as heretofore in similar cases, by broken lines.

For the optimal series, the area of slight injury (marked by small circles) occupies the entire left central portion of the triangle (fig. 10), extending from base to apex and reaching the left margin at culture R8C1. The area of severe injury (marked by crosses) lies along the left margin of the triangle, including all the cultures on this margin excepting R8C1, and extending to the right, at the lower left angle of the triangle, to include culture R1C2. On the supra-optimal triangle (fig. 11), the area of slight injury occupies a position corresponding to that of the same area on the optimal triangle. The area of severe injury in this series occupies the same region as in the optimal diagram, but extends farther to the right in its lower portion, so as to include cultures R1C2, R2C2, and R3C2.

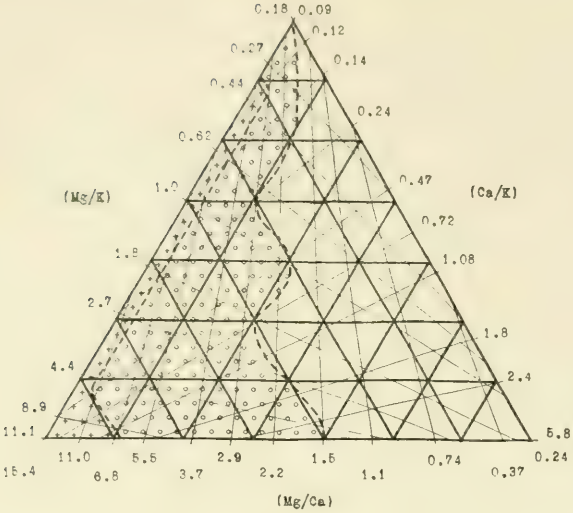

FIG. 10. Triangular diagram for optimal total concentration (1.75 atm.), showing the solutions producing no leaf injury (unshaded), those giving only slight leaf injury (indicated by small circles), and those giving severe leaf injury (indicated by crosses).

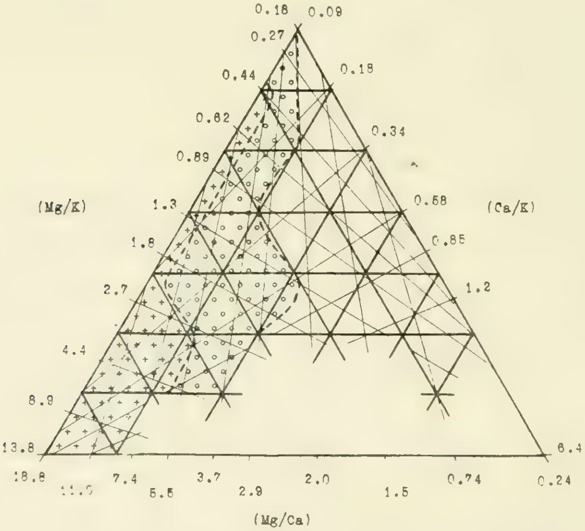

FIG. 11. Triangular diagram for supra-optimal total concentration (4.00 atm.) showing relation of salt proportions to leaf injury, as in figure 10.

A study of the diagrams of figures 10 and 11 brings out the fact that the severely injured cultures have osmotic partial concentrations of $Ca(NO_3)_2$ varying from 0.1 (R1C1) to 0.2 (R1C2), but culture R8C1, in which this concentration has also a value of 0.1, was not severely injured. It is thus clear that the partial osmotic concentration of $Ca(NO_3)_2$ is not a clearly limiting factor in the production of this injury. Similarly, the injured cultures have osmotic partial concentrations of $MgSO_4$ varying from 0.2 (R7C1) to 0.8 (R1C1), while culture R8C1, not seriously injured, exhibits this partial concentration as 0.1. From this it appears that an osmotic partial concentration of $MgSO_4$ as low as 0.1 does not produce severe injury. Nevertheless, it is clear that values of this partial concentration from 0.2 to 0.6 may or may not be severely injurious, according to other conditions. Finally, the osmotic partial concentration of KH_2PO_4 may have any value from 0.1 to 0.7 without producing severe leaf injury, and also, for the same range, it may produce injury. The only value of this partial concentration not ever accompanied by pronounced injury is 0.8 (R8C1). From these considerations it may be concluded that severe injury is definitely absent only when the osmotic partial concentration of $Ca(NO_3)_2$ is higher than 0.2, when that of $MgSO_4$ is lower than 0.2, and when that of KH_2PO_4 is higher than 0.7. It is thus obvious that the conditions determining severe injury must be related to the relative proportions of the three salts rather than to osmotic partial concentration of any one salt, a conclusion already reached by Tottingham for his similar series of cultures.

Considering the ratios of the osmotic partial concentrations, as was done by Tottingham, it appears that severe injury accompanies the ratio $MgSO_4/Ca(NO_3)_2$ when this ratio has a value between 2.0 (R7C1) and 8.0 R1C1. For the remainder of the series, not severely injured the value of this ratio varies from 1.0 (R8C1, R6C2) to 3.0 (R2C2, optimal). This makes it clear that no severe injury is produced with these ratio values below 2.0, and that severe injury always occurs when these values are greater than 3.0. Osmotic ratio values of $MgSO_4/Ca(NO_3)_2$ from 2.0 to 3.0 are thus questionable, and may or may not produce severe injury, apparently according to other conditions. The highest value of this ratio with which no injury occurred is 1.33 (culture R3C4). If therefore seems that no injury is to be expected, in 3-salt solutions such as were here employed, with osmotic ratios of $MgSO_4/Ca(NO_3)_2$ whose values are less than 1.33. Tottingham (page 203) has placed the limiting value of this ratio at 0.25, and notes that osmotic ratios of $MgSO_4/Ca(NO_3)_2$ with values of 2.0 or above may be expected to produce marked injury in his 4-salt solution.

A consideration of the cation ratio values with reference to leaf injury brings out some interesting features. The ranges of injury indicated on the diagrams of figures 10 and 11 are seen at once to bear no definite relation to either of the ratios Mg/K or Ca/K; these regions embrace all or nearly all of the total ranges of the values of both these ratios. On the other hand, it is at once obvious that solutions characterized by values of the ratio Mg/Ca below 1.5 give plants without injury in the optimal series, and those where this ratio value is below 2.2 give uninjured plants in the supra-optimal series. From these considerations it appears that the limit of slight injury is practically set by the magnitude of the ratio of magnesium to calcium. The limit of severe injury is not so readily defined; it surely is related to other properties of the solution than the Mg/Ca ratio.

Condition of roots

No great differences between the roots of the various cultures of the sub-optimal series were apparent. The roots of the optimal and supra-optimal series, however, showed marked differences even during the first ten days of their growth. During the first twelve days after they were placed in the nutrient solutions, the roots of cultures R1C1, R1C2, R2C1, R3C1, R4C1, R5C1, R6C1, and R7C1, were distinguished from the remaining cultures of these series by the absence of secondary roots and by short, thickened primary roots. During the later stages of growth numerous lateral roots appeared in these cultures, but took the form of short, stubby outgrowths. In cultures R1C1, R2C1, R3C1, and R4C1, these numerous lateral roots assumed the form of small, rounded tubercles. This latter condition of the roots was coincident with the most severe injury to the leaves, and injured roots were confined to the areas of severe leaf injury on the diagrams of figures 10 and 11. The root systems of cultures characterized by slight leaf injury appeared to be particularly *well* developed, although the lateral roots of cultures R1C3 and R2C2 were somewhat shorter than those of the other cultures with slight leaf injury. In cultures where leaf injury was not apparent at all, root development did not appear to be so vigorous, in general, as in those suffering slight leaf injury. It thus appears that the most vigorous root growth (for optimal and supra-optimal total concentrations) may be expected to occur in solutions whose osmotic ratio $MgSO_4/Ca(NO_3)_2$ is somewhat above 1.33. The partial concentration of KH_2PO_4 appears to have no influence upon the plants, as far as either leaf or root injury is concerned.

4. TRANSPIRATION DATA

Transpiration as a measure of growth

Introductory. Transpiration being a process that is going on at all times in the living plant, one to which many other plant processes are surely related, and also one that is somewhat readily measured, it has seemed worth while to study the relative amounts of water lost from the various cultures during their growth and to compare these transpiration data with the results of the other plant measurements.

The relative rate of water loss has been emphasized by several writers as an approximate measure of plant growth. Thus Whitney and Cameron[22] and other workers with plant cultures in the U. S. Bureau of Soils,[23] have taken the total amount of transpirational water loss during the growth of a culture as being more or less directly proportional to the growth of the plant. The relation between the amount of transpiration, dry weight, leaf surface, etc., for cultures of wheat during the first few weeks of their growth, was studied by Livingston,[24] who came to the conclusion that in many instances the amount of water lost by plants of the same species and of the same age, grown in the same locality but in different soils or solutions, is roughly proportional to the extent of leaf surface, and therefore, to the size of the plants. Of course such a relation can be expected to hold only where the relative amounts of water lost by transpiration are determined only by the size of the plants, not by internal physiological conditions. If the plants differ internally in some manner that affects transpiration otherwise than by differences in size, then transpiration should not be a measure of the size, although it might still be a valuable indicator of the general health of the plants. The general complexity of the problem thus suggested, as to whether transpiration may be reasonably employed to indicate relative growth and health of plants, apparently furnishes the explanation for Harris's[25] unexplained remark that Livingston's data do not justify his conclusions in this regard.

The transpiration diagrams. As has been remarked, the amount of water lost from each culture during the period of its growth was determined by summing the various losses recorded during that period. The total loss thus obtained for each culture was then expressed in terms of the total loss for culture R1C1 of the same series, considered as unity. The relative loss thus obtained for each culture of series A and that for the corresponding culture of series B, were averaged in all cases.

Table IX presents the transpiration data in three sections, each section referring to a single total concentration of the medium. The first column of the table gives, as heretofore, the culture or solution numbers referring to the triangular diagrams. In each of the three sections of the table (sub-optimal, optimal, and supra-optimal), the first two columns give the losses, relative to the loss from culture R1C1 in each case, for series A and B, respectively. The third column of each section gives the average losses for series A and B together, also relative to the average loss for culture R1C1.

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[22] Whitney, M. and Cameron, F. K., Investigations in soil fertility. U. S. Dept. Agric. Bur. Soils Bull. 23. 1904.

[23] Livingston, B. E., Britton, J. C., and Reid, F. R., Studies on the properties of an unproductive soil. U. S. Dept. Agric. Bur. Soils Bull. 28. 1905.

Livingston, B. E., Further studies on the properties of unproductive soils. U. S. Dept. Agric. Bur. Soils Bull. 36. 1907.

[24] Livingston, B. E., Relation of transpiration to growth in wheat. Bot. Gaz. **40:** 178–195. 1905.

[25] Harris, F. S., Effects of variations in moisture content on certain properties of a soil and on the growth of wheat. Cornell Univ. Agr. Exp. Sta. Bull. 352. 1914.

TABLE IX

Transpiration data for wheat grown 23 days in 3-salt solutions; series A, conducted from September 8 to October 1, series B, from November 13 to December 6, 1914.

CULTURE NUMBER	SUB-OPTIMAL TOTAL CONCENTRATION (0.1 ATM.)			OPTIMAL TOTAL CONCENTRATION (1.75 ATM.)			SUPRA-OPTIMAL TOTAL CONCENTRATION (4.0 ATM.)		
	Series A	Series B	Average	Series A	Series B	Average	Series A	Series B	Average
R1C1	1.00*	1.00	1.00L	1.00	1.00	1.00L	1.00	1.00	1.00L
	(131)	(160)	(145)	(170)	(213)	(192)	(126)	(135)	(131)
C2	1.42	1.21	1.32	1.21	1.08	1.15	1.08	1.16	1.12
C3	1.41	1.41	1.41	1.17	1.12	1.15	....	....	....
C4	1.56	1.55	1.56	1.16	1.19	1.18	....	....	....
C5	1.78	1.27	1.53	1.19	1.14	1.17	....	....	....
C6	1.91	1.57	1.74H	1.20	1.07	1.14	....	....	....
C7	1.61	1.47	1.54	1.18	1.14	1.16	....	....	....
C8	1.78	1.44	1.61H	1.15	1.14	1.15	1.06	1.35	1.21
R2C1	1.22	1.16	1.19L	1.07	1.08	1.08	0.97	1.07	1.02L
C2	1.38	1.35	1.37	1.14	1.11	1.13	1.11	1.16	1.14
C3	1.58	1.49	1.54	1.21	1.09	1.15	1.07	1.40	1.24H
C4	1.79	1.51	1.65H	1.15	1.08	1.12	....	....	....
C5	1.59	1.58	1.59H	1.02	1.13	1.08	....	....	....
C6	1.57	1.66	1.61H	1.15	1.17	1.16	1.15	1.33	1.24H
C7	1.91	1.46	1.69H	1.12	1.08	1.10	....	....	....
R3C1	1.48	1.18	1.33	1.17	0.99	1.08L	0.86	1.15	1.01L
C2	1.69	1.50	1.60H	1.31	1.09	1.20H	1.09	1.27	1.18
C3	1.66	1.54	1.60H	1.35	1.19	1.27H	1.15	1.36	1.26H
C4	1.86	1.67	1.77H	1.14	1.15	1.15	1.14	1.27	1.21
C5	1.78	1.51	1.65H	1.16	1.14	1.15	1.13	1.27	1.20
C6	1.80	1.64	1.72H	1.15	1.16	1.16	1.15	1.31	1.23H
R4C1	1.36	1.19	1.28	1.15	1.07	1.11	1.06	1.16	1.11
C2	1.74	1.34	1.54	1.21	1.08	1.15	1.08	1.31	1.20
C3	1.75	1.48	1.62H	1.18	1.09	1.14	1.23	1.38	1.31H
C4	1.66	1.50	1.58H	1.24	1.09	1.17	1.15	1.34	1.25H
C5	1.86	1.59	1.73H	1.24	1.27	1.26H	1.22	1.34	1.28H
R5C1	1.37	1.19	1.28	1.14	1.16	1.15	1.00	1.13	1.07L
C2	1.58	1.36	1.47	1.34	1.20	1.27H	1.14	1.30	1.22
C3	1.66	1.37	1.52	1.24	1.14	1.19H	1.07	1.36	1.17
C4	1.53	1.34	1.44	1.28	1.06	1.17	1.19	1.26	1.23H
R6C1	1.45	1.20	1.33	1.23	1.13	1.18	1.05	1.18	1.12
C2	1.70	1.38	1.54	1.21	1.13	1.17	1.05	1.24	1.15
C3	1.74	1.44	1.59H	1.23	1.08	1.16	1.05	1.22	1.14
R7C1	1.50	1.21	1.36	1.19	1.07	1.13	0.98	1.10	1.04L
C2	1.71	1.38	1.55	1.28	1.21	1.25H	1.03	1.16	1.10
R8C1	1.47	1.17	1.32	1.24	1.00	1.12	1.09	0.96	1.03L
K**	1.84	1.57	1.71	1.16	1.07	1.12	0.99	1.04	1.02
T**	1.54	1.51	1.53	1.23	1.22	1.23	1.18	1.19	1.19

* The loss for culture R1C1 is always taken as unity, and the other losses are expressed in terms of this. The actual loss for culture R1C1 is given in parenthesis, in each case, in cubic centimeters.

** K and T represent Knop's solution and Tottingham's best solution, respectively: these data are introduced for comparison.

The letters L and H, following the average data as in the tables III, IV and V, represent numbers lying, respectively, within the lower and within the upper one-fourth of the total range of the series of averages.

The average data of table IX were plotted on the triangular diagrams, as were those of the dry weight yields, and the diagrams thus obtained were compared with the yield diagrams already given (figs. 2–4 and 6–8). Since the notations L and H of table IX indicate the low and high regions of these transpiration diagrams, the latter need not be here presented. The following observations obtained by comparing them with the corresponding yield diagrams may be given, however.

On the sub-optimal diagram the region of low transpiration embraces only the extreme lower portion (culture R1C1 and R2C1) of the region of low yields of tops (fig. 2). The region of high top yields lies entirely within that of high transpiration, but the latter region is considerably larger than the former. These two diagrams agree, in general, in showing the lower top yields and lower transpirations as occupying the left side of the triangle, while the higher yields and transpirations occupy the middle and lower right. About as good agreement is manifest between the diagrams of root yields (fig. 6) and that of transpiration, in the sub-optimal series. It is to be emphasized here, however, that *low* transpiration corresponds to *high* yields of roots, while *high* transpiration corresponds to *low* yields of roots.

In the optimal series, there is an exceedingly close agreement between transpiration and yield of tops (fig. 3), both for the regions of low and for those of high values. Here the amount of water transpired during the growth of the plants appears to be as good a criterion as is the final dry weight, for judging the comparative growth obtained in the different solutions. Comparison of the transpiration diagram with that of root yields for the optimal series (fig. 7), shows that there is here no detailed agreement. All that may be said is that the *low* transpiration data agree, in a general way, with the data of *high* root yields, by lying to the left of the region of *high* transpiration and and of *low* root yields.

The supra-optimal diagram of transpiration agrees very closely, but not quite exactly, with the diagram of top yields (fig. 4); the agreement is exact in the case of the regions of low values. No general agreement is to be noted between the diagram of transpiration and that of root yields (fig. 8) in the supra-optimal series, though the culture giving highest root yields also gave highest transpiration.

From the last three paragraphs it appears that the amount of water lost by the plants is generally a valuable indication of the top yields, under the conditions of these cultures, low transpiration corresponding to low yield of tops and high transpiration to high yield. This is especially true of the optimal and supra-optimal total concentrations. It also appears that there is no marked correlation between yield of roots and transpiration, though the sub-optimal and optimal series agree in showing a general, but inverse, correspondence in this regard. For the sub-optimal series it appears that *high* transpiration is correlated with low root yields.

Relation of transpiration to total concentration of the medium. To bring out the relations between the amounts of water lost by transpiration and the total concentration of the nutrient solution, graphs of the average transpiration data for each of the three series were prepared, in a manner quite similar to that employed for the study of top and root yields (figs. 5 and 9). These three transpiration graphs are shown in figure 12. The actual losses for the optimal series are here arranged in the order of their magnitudes, from the highest to the lowest, and are then plotted to form the nearly straight line of the figure. With the various cultures arranged in the order determined by this graph the two other series are plotted on the same scale. These three graphs show clearly that there is no general tendency for transpiration to be either higher or lower with the sub-optimal than with the optimal total concentration. On the other hand, it is plain that none of the transpiration data for the supra-optimal series is nearly as great as are the corresponding data for the sub-optimal and optimal series. This latter relation was brought out in the study of the relation of top yields to total concentration (fig. 5), but in that case a pronounced and general difference is observed between the top yields of the sub-optimal and optimal series. Since no such difference is here manifest, it is clear that the criterion of transpiration, while valuable as indicating large differences in growth (without the destruction of the plants), is not always as definite in its indications as is the criterion of dry weight of tops.

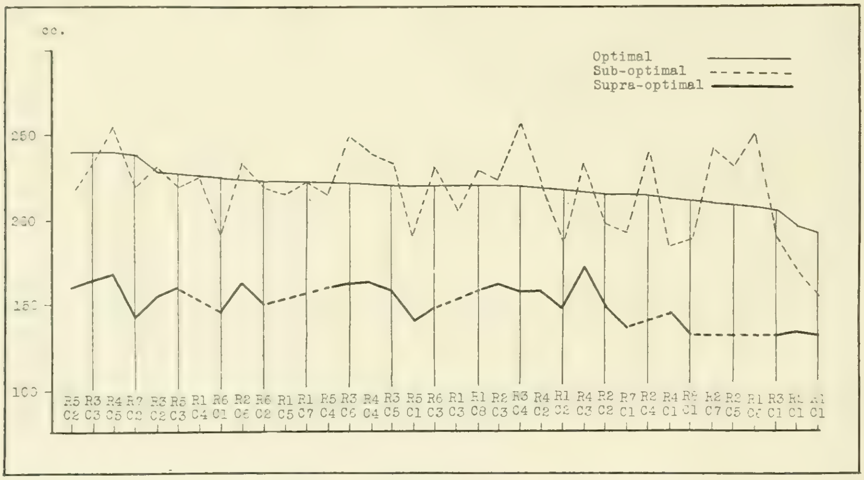

FIG. 12. Amounts of water lost by transpiration (cubic centimeters) from wheat plants grown with sub-optimal, optimal, and supra-optimal total concentrations.

Water requirement

Introductory. The relations between the amounts of water lost by transpiration and the dry weight yields of the various cultures may be studied by means of the ratios of transpiration to yield. Such ratios represent the water requirements of the plants, being the amount of water required, in each case, to produce a single gram of yield.

Apparently the first worker to give attention to water-requirement as a measurable physiological character of plants was Woodward,[26] whose work was carried out over 215 years ago. This author found that plants grown in water with low salt content had a much higher water requirement (per unit of *green* weight) than those grown in water containing more dissolved salt. Many other experimenters have studied the water requirements of plants grown under various conditions, although water cultures have been employed in but little of this work. Physiologists do not seem generally to have realized the importance of this criterion of plant growth, and most of the experiments reviewed in Briggs and Schantz's[27] excellent and exceedingly useful summary of the literature of this subject were carried out by agriculturists, and with only approximate control of the various effective conditions. Sorauer's experiments with water cultures may, however, be mentioned here, and also those of Heinrich.

Sorauer[28] studied rye, barley, wheat and oats, grown 53 days in "normal" ([1885], page 87) nutrient solutions of different total concentrations (from 0.05 to 1.00 "per cent") and found that the water requirement (per unit of dry yield) decreased with increase in the total concentration of the medium. Thus, according to Briggs and Schantz's ([1913], page 55) computations of Sorauer's results, the water requirement for wheat in these tests was 768 ± 40 when the total concentration of the solution was 0.05 per cent, and only 469 ± 18 when the total concentration was 0.50 per cent.

Heinrich[29] grew oats to maturity in nutrient solutions containing $4H_2KPO_4 + CaCl_2 + 5Ca(NO_3)_2 + 2MgSO_4 + 2Fe$ (Briggs and Schantz [1913], page 55), with total concentrations varying from 0.1 g. to 3.0 g. per liter. The weakest solution showed a water-requirement (per unit of dry yield) of 642 and the next to the weakest (0.25 g. per liter) gave a corresponding value of 688. Throughout the remainder of the series the water-requirement decreased as the total concentration of the medium increased, and the value obtained for the plants grown in the solution containing 3.0 g. per liter was 515. Thus, with the exception of the very weakest solutions (where the plants were obviously unhealthy), the water requirement is less with more concentrated solutions and greater with weaker ones, which is in general agreement with the conclusion reached by Sorauer.

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[26] Woodward, J., Some thoughts and experiments concerning vegetation. Phil. Trans. Roy. Soc. London **21:** 193–227. 1699.

[27] Briggs, L. J., and Shantz, H. L., The water requirement of plants. II. A review of the literature. U. S. Dept. Agric. Bur. Plant Ind. Bull. 285. 1913.

[28] Sorauer, P., Nachtrag zu den "Studien über Verdunstung." Forsch. Geb. Agric. Phys. **6:** 79–96. 1883.

[29] Heinrich, R., Ueber die Wassermengen, welche die Haferpflanze aus verschiedenen Nährstoff-Concentrationen während ihrer Vegetationszeit verbraucht. Zweiter Bericht über die Verhältnisse und Wirksamkeit der Landwirtschaftlichen Versuchs-Station, Rostock. Pages 179–174. 1894.

In the present case the average amount of water lost by transpiration from each culture in series A and B was divided by the corresponding yield of tops and also by the corresponding yield of roots. The water requirement ratios thus obtained, for tops and for roots, expressed in terms of the corresponding ratio for culture R1C1, are set forth in table X. The actual average water requirements (grams of water required to produce a single gram of dry material) are given for culture R1C1, in each case, this number being placed in parenthesis below the relative value (unity) of culture R1C1 in the comparative series. The letters L and H indicate, as in previous tables, that the numbers opposite which they are placed fall within the lower one-fourth and within the upper one-fourth, respectively, of the total range of their respective series.

Relative water requirements. A study of the six triangular diagrams prepared from the data of table X brings out several interesting suggestions, but no precise generalizations can be made in this connection, and the diagrams will not be given here. It may, however, be worthy of mention that, in the optimal series, low water requirement of tops corresponds, in a general way, with high top yield, while in the sub-optimal series low water requirement of roots corresponds with low top yield. These diagrams emphasize the fact that optimum physiological balance, or the best salt proportions, of the nutrient medium, to give either low or high water requirement ratios, does not occur in the same solutions in the three different series. How a given set of salt proportions affects the plant in this respect is determined by the total concentration of the mixture.

Relation of total concentration to the actual magnitudes of the water requirement ratios. As in the cases of yields of tops and roots and of transpiration (figs. 5, 9 and 12), the actual water requirements of tops and of roots have been graphically represented in a linear way, and these graphs are given in figures 13 and 14. In both cases the data for the optimal series were arranged in a decreasing order and then plotted as ordinates, with arbitrary, equal abscissa increments representing the different solutions. The order of the solutions or cultures is thus not the same in figures 13 and 14. After this order was determined as the descending series for the optimal concentration, the corresponding data for the sub-optimal and supra-optimal concentrations were plotted on the same chart, in each case. These charts bring out the general relations between total concentration and water requirements of tops and of roots.

a. Water requirement of tops. For the water requirement of tops (fig. 13) it is seen at once that the total concentration of the medium determines the

TABLE X

Amount of water required for the production of each gram of yield of tops and of roots (water requirement), relative to that of culture R1C1. The data are averages from series A and B.

CULTURE NUMBER	SUB-OPTIMAL TOTAL CONCENTRATION (0.1 ATM.)		OPTIMAL TOTAL CONCENTRATION (1.75 ATM.)		SUPRA-OPTIMAL TOTAL CONCENTRATION (4.0 ATM.)	
	Tops	Roots	Tops	Roots	Tops	Roots
R1C1	1.00L* (559)	1.00L (1407)	1.00 (466)	1.00L (1802)	1.00L (363)	1.00 (1506)
C2	1.08L	1.23L	0.96	1.03L	1.00L	1.09H
C3	1.13	1.34	0.95	1.22	....	....
C4	1.20	1.71	1.02H	1.10	....	....
C5	1.20	1.74	0.92L	1.17	....	....
C6	1.25H	1.76	0.94	1.15	....	....
C7	1.23H(?)	1.98H	1.06H	1.15	....	....
C8	1.28H	1.99H	0.99	1.21	1.07	1.11H
R2C1	1.16	1.00L	1.00	1.07L	1.05	0.93
C2	1.10	1.31	1.00	1.07L	1.00L	1.01
C3	1.11	1.65	0.92L	1.24H	0.97L	1.05H
C4	1.11	1.76	0.89L	1.18	....	....
C5	1.13	2.07H	0.91L	1.21	....	....
C6	1.12	2.13H	0.96	1.19	0.96L	1.05H
C7	1.22	2.09H	0.91L	1.07L	....	....
R3C1	1.21	0.95L	0.93L	1.06L	0.96L	0.92
C2	1.24H	1.34	0.96	1.12	1.05	1.02
C3	1.13	1.51	0.93L	1.18	1.00L	1.07H
C4	1.12	1.86H	0.90L	1.21	0.97L	1.10H
C5	1.06L	1.94H	0.92L	1.25H	0.96L	1.02
C6	1.31H	1.90H	1.00	1.25H	1.10H	1.00
R4C1	1.28H	0.97L	0.99	1.07L	1.04	0.87L
C2	1.26H	1.24L	0.90L	1.05L	0.97L	0.92
C3	1.13	1.53	0.90L	1.25H	0.95L	0.98
C4	1.05L	1.85H	0.91L	1.29H	1.02	0.98
C5	1.06L	1.97H	0.98	1.21	1.02	1.03
R5C1	1.21	1.00L	0.96	1.08L	1.08H	0.84L
C2	1.03L	1.26	0.91L	1.18	1.01	0.99
C3	1.08L	1.69	0.96	1.29H	1.00L	1.07H
C4	1.12	2.05H	0.92L	1.14	1.02	1.00
R6C1	1.22	1.01L	1.01	1.11	1.07	0.87L
C2	1.03L	1.41	0.99	1.25H	0.98L	0.94
C3	1.11	1.64	0.96	1.32H	1.05	1.01
R7C1	1.26H	0.98L	0.98	1.10	1.03	0.84L
C2	1.18	1.18	0.95	1.23	1.05	0.90L
R8C1	1.19	0.96L	1.03H	1.10	1.13H	0.86L
K**	1.29	1.94	1.02	1.18	0.98	1.02
T**	1.02	1.66	0.96	1.20	0.97	0.95

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* The water requirement for culture R1C1 is always taken as unity, and the other water requirements are expressed in terms of this. The actual water requirement for culture R1C1 is given in parenthesis, in each case.

** K and T represent Knop's solution and Tottingham's best solution respectively; these are introduced for comparison.

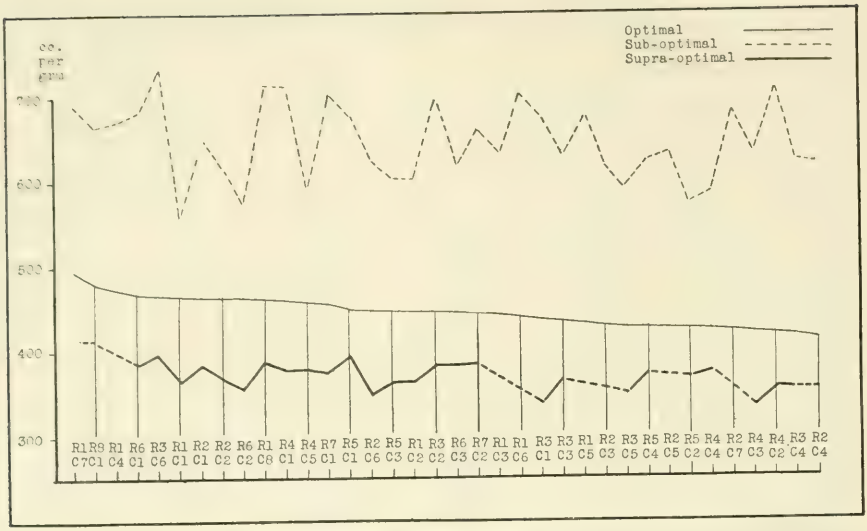

FIG. 13. Water requirement (cubic centimeters) per gram of wheat tops grown in sub-optimal, optimal, and supra-optimal total concentrations.

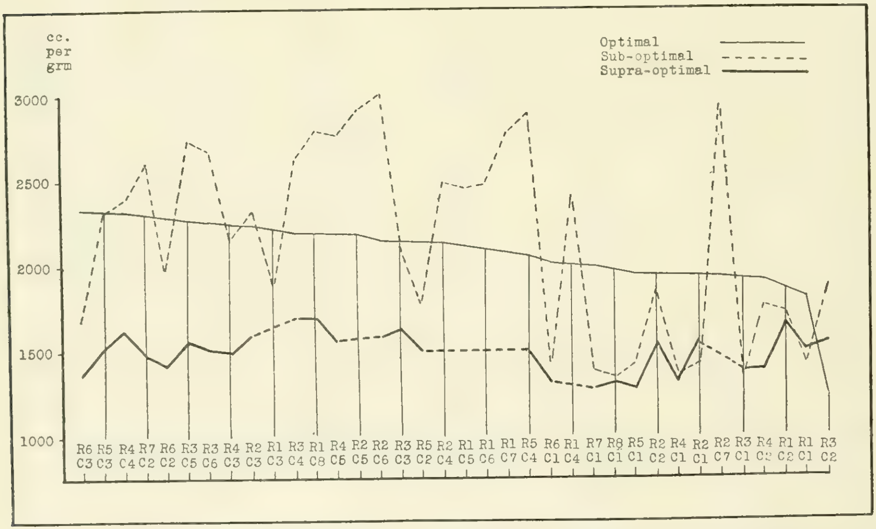

FIG. 14. Water requirement (cubic centimeters) per gram of wheat roots grown in sub-optimal, optimal, and supra-optimal total concentrations.

general position of the graph. There is here no crossing of the graphs; the highest water requirements of tops are all shown as occurring in the sub-optimal series, the lowest requirements are those of the supra-optimal series, and the optimal series exhibits water requirements of tops that occupy an intermediate position. A clear generalization is here indicated: the higher the total concentration the lower is the amount of water required for the production of a gram of dry tops. This conclusion is perfectly definite, for the conditions of the present study at least, and appears to agree with what might be expected from an *a priori* consideration of the problem in hand. It appears that water loss by transpiration is retarded more by high concentration of the nutrient medium than is the production of yield of tops, and it seems quite clear that this is due to a physical (rather than to a chemical) property of the solution, being probably related directly to osmotic phenomena. With more concentrated solutions the resistance to water absorption by the roots is increased, and this resistance, in turn, decreases the transpiring power[30] of the plants.

The arrangement of these three graphs of water requirements of tops is entirely different from that of the graphs of dry weights of tops (fig. 5). The optimal total concentration gives highest yields of tops and medium water requirements of tops, the supra-optimal concentration gives medium yields of tops and lowest water requirements, and the sub-optimal concentration gives lowest yields and highest water requirements. It appears that a very important generalization (as far as the water relations of plants are concerned) is here touched upon, one that will surely repay more thorough study.

b. Water requirement of roots. The graphs of figure 14 indicate that there is no clear relation between water requirement of roots and total concentration of the medium, for the sub-optimal and optimal total concentrations. On the other hand, these graphs do show a very clear relation, as to the water requirements of roots, between the optimal and supra-optimal total concentrations. The root water requirement is almost always markedly lower, for any set of salt proportions, in the supra-optimal solutions than in the optimal ones. The relations are here about the same as for the water requirements of tops, as far as these two higher total concentrations are concerned, and the same condition holds in comparison with transpiration (fig. 12). In a general way it may be said that the root water requirements for the sub-optimal concentration are lower and more nearly like those for the optimal concentration than is the case with the water requirements of tops.

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[30] Livingston, B. E., and Hawkins, Lon. A., The water-relation between plant and soil. Carnegie Inst. Wash. Pub. **204**: 3–48. 1915.

Pulling, H. E., and Livingston, B. E., The water-supplying power of the soil as indicated by osmometers. Carnegie Inst. Wash. Pub. **204**: 49–84. 1915.

Comparison of figures 5, 12, 13 and 14, shows that the relation between the optimal and supra-optimal total concentrations is much the same, whether this relation is judged by the criterion of dry weight of tops, by that of transpiration, by that of water requirement of tops, or by that of water requirement of roots.

5. GENERAL COMPARISON OF QUANTITATIVE PLANT DATA

Relation between the areas of low, medium, and high relative plant values, as shown on the various triangular diagrams

The quantitative plant data that have been presented in the preceding sections (those of yield of tops, yield of roots, transpiration, water requirement of tops and water requirement of roots) have been studied with reference to their distribution into areas of low, medium, and high values on the triangular diagrams. A range of total concentration—the physical property of the nutrient medium—from 0.1 atmosphere to 4.0 atmospheres has been studied, this total range being represented in the experiments by the limiting concentrations just mentioned and by an optimal concentration of 1.75 atmospheres. For each kind of plant measurement three triangular diagrams have thus been required, each diagram corresponding to one of these three total concentrations. The chemical character of the nutrient medium,—the proportions of the three main component salts,—has been studied by varying the salt proportions, in each of the three total concentrations, from osmotic proportions of 1 : 1 : 8 to those of 1 : 8 : 1 and to those of 8 : 1 : 1, the increments of variation being always one-tenth of the total diffusion tension or possible osmotic pressure of the solution as a whole. The total range of salt proportions has thus been represented by 36 different sets of proportions in each case, each set of proportions having its particular location on the triangular diagram. The position of a given solution on the diagram thus characterizes it as having a definite set of salt proportions (ion ratio values, etc.), or as having a certain complex of *chemical* properties, and the particular one of the three diagrams on which a given solution is located characterizes it as having a certain *physical* character determined by its total concentration.

It is of course clear that salt proportions other than those actually employed might have been tested, and that each of these would have had a definite location on the triangle. It is also obvious that total concentrations other than the three here employed might have been included in the experiments, and each one of these would have called for a separate triangle of its own. The whole system of possible variations in the chemical and physical properties of the nutrient solutions here dealt with may be pictured by means of a solid diagram, a right prism with an equilateral triangle as base. Passing from below upward, each successive horizontal (triangular) section of this prism may represent a different total concentration of the nutrient solution, and each vertical line passing through the prism (thus cutting all the horizontal triangular sections) may represent a different set of salt proportions.

The various average values obtained for the five different plant measurements considered have been studied by plotting them on their respective diagrams, always expressing each value in terms of that for solution R1C1 of the same diagram, and the area of each diagram has been separated into three portions, designated as the areas of low, medium, and high values. Values of any plant measurement that lie within the lower one-fourth of the total range of that particular measurement, for that particular total concentration, have been termed low values; those lying within the upper one-fourth have been termed high values; and all other values for that kind of measurement and for that total concentration have been termed medium values. This notation simplifies the problem of comparing a large number of measurements, and the grouping of the thirty-six different values into but three classes should tend to avoid many complications arising from smaller variations in the values, brought about by the influence of unknown conditions upon the growth and development of the plants.

To compare all the different plant measurements and to relate them to the chemical and physical conditions of the solutions in which the plants were grown presents a somewhat complicated problem, which has been attacked only in part in the foregoing discussions. For each kind of plant measurement it is necessary to determine how the value alters with its position on the triangle and also how it alters with the position of the triangle in the prismatic diagram. The sub-optimal diagram of these studies may be considered as the lower base of the prism in question, the supra-optimal triangle being the upper base, while the optimal triangle is a horizontal section of the prism between the two bases. Each kind of plant measurement requires a prism of its own and a not unimportant part of the interpretation of the results involves comparisons between similar areas or points in the different prismatic diagrams.

Some of these various and complicated relations have been brought out in the foregoing discussions and still others will be evident from the tabular summary of all quantitative plant measurements presented in table XI. This table shows the relative value (low, medium, or high, denoted by the letters L, M and H) of each average plant measurement (series A and B combined) for each of the three different total concentrations and for each of the thirty-six different sets of salt proportions. The salt proportions are denoted by the solution numbers, in the first column, and the total concentrations are shown by the words sub-optimal, optimal, and supra-optimal, as heretofore. Table XI is virtually a summary of all the triangular diagrams, and of the columns of averages given in tables III, IV, V, IX, and X, in terms of low, medium and high values. From it may be determined the relations

TABLE XI

Summary of growth measurements of plants grown in 3-salt solutions of 36 different sets of salt proportions and of three different total concentrations, in terms of low (L), medium (M), and high (H) values, from tables III, IV, V, IX, and X.

	TOP YIELDS			ROOT YIELDS			TRANSPIRATION			WATER REQUIREMENTS					
										Tops			Roots		
Sol. No.	Sub-optimal	Optimal	Supra optimal	Sub-optimal	Optimal	Supra optimal	Sub-optimal	Optimal	Supra optimal	Sub-optimal	Optimal	Supra optimal	Sub-optimal	Optimal	Supra optimal
R1C1	L	L	L	M	M	L	L	L	L	L	M	L	L	L	M
C2	M	M	M	M	H	L	M	M	M	L	M	L	L	L	H
C3	M	M	—	M	L	—	M	M	—	M	M	—	M	M	—
C4	M	M	—	M	H	—	M	M	—	M	H	—	M	M	—
C5	M	M	—	L	M	—	M	M	—	M	L	—	M	M	—
C6	M	M	—	L	M	—	H	M	—	H	M	—	M	M	—
C7	M	M	—	L	M	—	M	M	—	H	H	—	H	M	—
C8	M	M	M	L	M	M	H	M	M	H	M	M	H	M	H
R2C1	L	L	L	M	M	M	L	M	L	M	M	M	L	L	M
C2	M	M	M	M	M	M	M	M	M	M	M	L	M	L	M
C3	M	M	H	M	L	M	M	M	H	M	L	L	M	H	H
C4	M	M	—	M	M	—	H	M	—	M	L	—	M	M	—
C5	M	M	—	L	L	—	H	M	—	M	L	—	H	M	—
C6	M	M	H	L	M	M	H	M	H	M	M	L	H	M	H
C7	M	M	—	L	M	—	H	M	—	M	L	—	H	L	—
R3C1	L	M	M	H	M	M	M	L	L	M	L	L	L	L	M
C2	M	M	M	M	H	M	H	M	M	H	M	M	M	M	M
C3	M	H	H	M	H	M	H	H	H	M	L	L	M	M	H
C4	H	M	M	M	M	M	H	M	M	M	L	L	H	M	H
C5	H	M	H	L	L	M	H	M	M	L	L	L	H	H	M
C6	M	M	M	M	L	M	H	M	H	H	M	H	H	H	M
R4C1	L	M	M	H	M	H	M	M	M	H	M	M	L	L	L
C2	M	M	M	H	H	H	M	M	M	H	L	L	L	L	M
C3	M	M	H	M	L	H	H	M	H	M	L	L	M	H	M
C4	H	M	M	L	L	H	H	M	H	L	L	M	H	H	M
C5	H	H	M	L	M	M	H	H	H	L	M	M	H	M	M
R5C1	L	M	L	H	H	H	M	M	L	M	M	H	L	L	L
C2	M	H	M	M	H	M	M	H	M	L	L	M	M	M	M
C3	M	M	M	M	L	M	M	H	M	L	M	L	M	H	H
C4	M	M	M	L	M	M	M	M	H	M	L	M	H	M	M
R6C1	L	M	M	H	H	H	M	M	M	M	M	M	L	M	L
C2	M	M	M	M	L	M	M	M	M	L	M	L	M	H	M
C3	M	M	M	M	L	M	H	M	M	M	M	M	M	H	M
R7C1	L	M	L	H	M	M	M	M	L	H	M	M	L	M	L
C2	M	H	M	H	M	M	M	H	M	M	M	M	M	M	L
R8C1	L	—	L	H	—	M	M	M	L	M	H	H	L	M	L
Total no. of L	8	2	5	11	10	2	2	2	6	8	14	13	10	9	6
M	24	29	18	17	16	20	19	28	14	20	19	12	15	19	15
H	4	4	5	8	9	6	15	6	8	8	3	3	11	8	7

holding between the different kinds of plant measurements for the same total concentration and also the relation holding between the same kind of plant measurements for different total concentrations and for different sets of salt proportions.

Table XII presents a correlation summary of all the quantitative plant data and shows the distribution of the high, medium, and low values obtained for each single criterion (yield, transpiration, and water requirement) as compared with those for the other criteria, the data being derived from those of table XI. The table as a whole is divided into an upper, middle and lower section, each of these sections referring to one of the three total concentrations here studied. It is divided vertically into four sections. The first of these presents the comparison between the yields of tops, on the one hand, and yields of roots, transpiration, and water requirements on the other. The second section gives the comparison between the root yields, on the one hand, and transpiration and water requirements on the other. The third section gives the comparison between transpiration, on the one hand, and the water requirement of tops and that of roots, on the other. The fourth and last section gives the comparison between the water requirement for tops and that for roots. The letters H, M and L, stand for high, medium, and low, as these terms have been used in table XI and in the preceding discussion.

The meaning of the numerical data of table XII is best brought out by examples. For the sub-optimal concentration, out of a total of four cultures giving high yields of tops, one gave medium, and three gave low yields of roots, all four gave high transpiration values and high water requirement for roots, and one gave medium and three low water requirement for tops. Similarly, of 24 cultures giving medium yields of tops, two gave high, 14 gave medium, and eight gave low yields of roots. All of the comparisons are stated in this manner. It will be seen that table XII may also be read in the vertical direction thus stating the comparison in the reverse order. For example, out of eight cultures with sub-optimal total concentration giving high root yields, two gave medium, and six gave low top yields. In order to bring out the dominant agreements and disagreements encountered in these comparisons the individual numerical data are marked in four different ways. Those numbers are printed in *italics* which represent more than half of the total number of cultures belonging in the group indicated by the letter (H, M, L) on the same line and at the *left* of that vertical section of the table. Thus, for the sub-optimal total concentration, the three cultures giving low root yields comprise more that one-half of the four cultures giving high top yields, and so this figure 3 appears in italics. Similarly, the 14 cultures giving medium root yields comprise more than half of the 24 giving medium top yields and the figure 14 is italicized. Reading the comparisons vertically, all those numbers that represent more than half of the total number of cultures belonging to the group indicated by the letter at the

TABLE XII

Relative distribution of high, low and medium dry weights, transpiration, and water-requirements for each of the three total concentrations here used, being a summary of the average data given in tables III, IV, V, IX, X and XI.

TOTAL CONCENTRATION	COMPARISON OF TOP YIELDS WITH ROOT YIELDS, TRANSPIRATION, AND WATER REQUIREMENT OF TOPS AND ROOTS													COMPARISON OF ROOT YIELDS WITH TRANSPIRATION AND WATER REQUIREMENT OF TOPS AND ROOTS										COMPARISON OF TRANSPIRATION WITH WATER REQUIREMENT OF TOPS AND ROOTS							COMPARISON OF WATER REQUIREMENT OF TOPS AND ROOTS			
	Yield tops	Yield roots			Transpiration			Water-requirement						Yield roots	Transpiration			Water-requirement						Transpiration	Water-requirement						Water req. tops	Water req. roots		
								Tops			Roots							Tops			Roots				Tops			Roots						
		H	M	L	H	M	L	H	M	L	H	M	L		H	M	L	H	M	L	H	M	L		H	M	L	H	M	L		H	M	L
Sub-optimal 0.10 atm.	H	—	1	3	4	—	—	—	1	3	4	—	—	H	—	8	—	3	5	—	—	1	7	H	4	8	3	9	6	—	H	3	2	3
	M	2	14	8	11	13	—	6	14	4	7	15	2	M	7	8	2	2	10	5	2	12	3	M	4	11	4	2	9	8	M	5	10	15
	L	6	2	—	—	6	2	2	5	1	—	—	8	L	8	3	—	3	5	3	9	2	—	L	—	1	1	—	—	2	L	3	3	2
Optimal 1.75 atm.	H	2	2	—	4	—	—	—	2	2	—	4	—	H	3	6	—	1	5	3	—	5	4	H	—	4	2	1	5	—	H	—	3	—
	M	7	12	10	2	26	1	2	15	12	8	15	7	M	2	12	2	1	9	6	—	11	5	M	3	14	11	7	14	7	M	4	9	6
	L	—	2	—	—	1	1	—	2	—	—	—	2	L	1	9	—	—	5	5	8	2	—	L	—	1	1	—	—	2	L	4	7	3
Supra-optimal 4.00 atm.	H	1	4	—	4	1	—	—	1	5	3	2	—	H	2	3	1	1	3	2	—	3	3	H	1	3	4	3	5	—	H	—	1	2
	M	4	13	1	4	13	1	1	10	7	4	11	3	M	6	10	4	2	9	9	6	11	3	M	—	7	7	4	7	3	M	1	7	4
	L	1	3	1	—	—	5	2	2	1	—	2	3	L	—	1	1	—	—	2	1	1	—	L	2	2	2	—	3	3	L	6	7	—

head of the column in which they occur are shown in black face type. Thus, for example, the six cultures giving low top yields comprise more than half of the eight giving high root yields, so that the figure 6 is in black type, but this same figure becomes also italicized because six is more than half of the eight cultures giving low root yields. Similarly, the figure 14 mentioned above as italicized is also in black face type, because 14 is more than half of the 17 cultures giving medium root yields. By this notation it is clear that the best correlations are indicated by black italics, where the agreement is good in both directions. Good agreement in the horizontal direction of comparison is indicated by italics and good agreement in the vertical direction is indicated by black type, which may be either italic or roman. Many of the points thus brought out have already been mentioned in the preceding discussions and space need not be here devoted to further consideration of these interesting agreements and disagreements.

Relation between the actual values for the various plant measurements

Finally, figure 15 is here presented to bring out the relations that hold between the actual values of the various plant measurements, which are represented graphically in much the same manner as that employed for figures 5, 9, 12, 13, and 14. In this case, however, the various different salt proportions (indicated at the base by the usual solution numbers) are all arranged in the decreasing order of their magnitudes for top yields of the optimal series. As in the previous graphs, a narrow line represents the optimal total concentration, a broad line represents the supra-optimal, and a narrow broken line represents the sub-optimal concentration. It will be seen that the figure shows five groups, each one of which comprises three graphs. The lower group (A, yield of roots) and the second group (B, yield of tops) are all plotted on the same scale and are directly comparable, as is indicated by the numbers at the left. The graphs of the third group (C, transpiration) are plotted on a different scale, which is indicated on the left margin. The fourth and fifth groups (D, water requirement per gram of tops, and E, water requirement per gram of roots) are all plotted on the same scale but the group E as a whole has been displaced downward in order to save space, as is clear from the ordinate values given at the left.

Inspection of figure 15 will bring out many of the relations between the various plant measurements, on the one hand, and the salt proportions and total concentration of the solution on the other, also between the different plant measurements for the same total concentration, the same set of proportions, etc. These graphs again emphasize the points already brought out, that the total concentration of the medium clearly determines the yield of tops (group B) and also the water requirement of tops (group D), for all salt proportions here tested; the order of magnitude with reference to total concentration is the same for all salt proportions in the case of either one of these

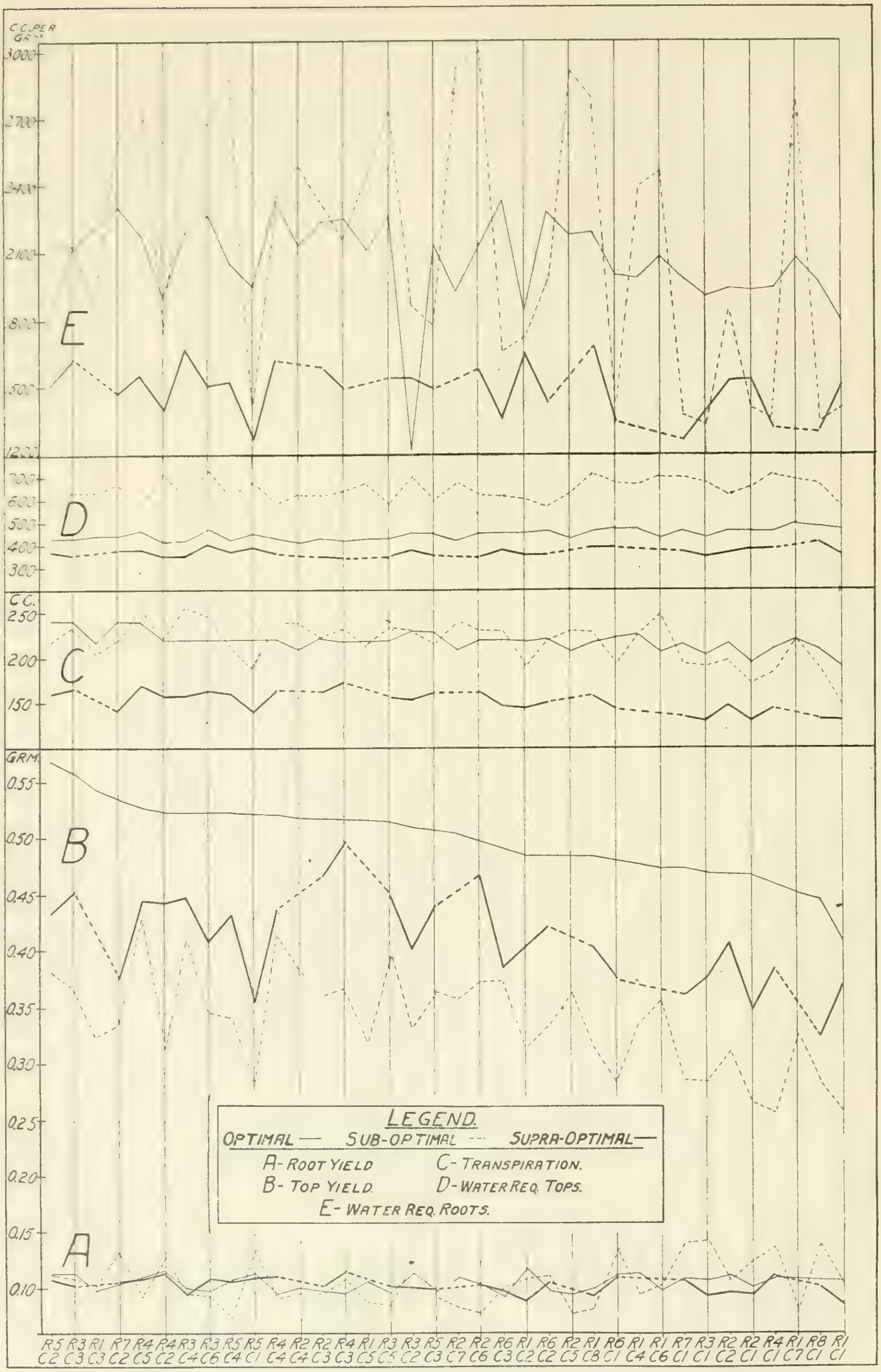

FIG. 15. Graphic summary of plant measurements.

two plant measurements, but the order for yield of tops is not at all the same as for water requirement of tops. The water requirement of tops is less as the total concentration of the medium increases, as has been found by other writers. The yield of tops, on the other hand, is greatest for the optimal, less for the supra-optimal, and least for the sub-optimal total concentration. Also, for transpiration (group C), the supra-optimal total concentration appears always to give the lowest value, for all the different sets of salt proportions, and the same generalization is true for nearly all the sets of salt proportions in the case of the water-requirement of roots (group E).

For yield of roots (group A) there is no general relation between the dry weights and the total concentration of the medium, but each particular set of salt proportions seems to have its own relations in this regard. Thus, solution R3C3 gives the highest root yield with the optimal total concentration, a medium yield with the sub-optimal, and the lowest with the supra-optimal. The same is true of solutions R5C2, R4C5, R1C4 and R2C2, but this is not true for any of the other sets of salt proportions. A similar shifting of the order of magnitude of the plant measurements, as related to the sub-optimal and optimal total concentrations, for different sets of salt proportions, is seen in the case of transpiration (group C) and water requirement of roots (group E), as has already been mentioned.

On the whole, the graphical summary of figure 15 shows clearly enough that the effect of any set of salt proportions is not, in general, relatively the same for different total concentrations, at least with the exception of the water requirement of tops, where there is considerable parallelism between the graphs for the optimal and supra-optimal series. It also shows that the effect of any total concentration is not generally the same for different sets of salt proportions. This summary shows, further, that root characters and top characters, of wheat grown as in these experiments, do not generally vary in the same way with reference to the conditions of the medium. Finally, it shows that different criteria for plant measurement may generally be expected to exhibit more or less pronounced differences in the relations between growth and the properties of the solution in which the plants are grown. Some of the details and exceptions to these generalizations are best brought out by study of the triangular diagrams previously given.

COMPARISON OF DRY WEIGHT YIELDS OF TOPS GROWN IN THE BEST SALT PROPORTIONS OF THE THREE-SALT SOLUTION, WITH THE CORRESPONDING YIELDS FROM SOLUTIONS EMPLOYED BY OTHER AUTHORS

As previously stated, Knop's solution and Tottingham's best solution for tops (T3R1C4) were included in each of the three experimental series here carried out, for purposes of comparison.

For the sub-optimal series (0.1 atm.) the highest average yield of tops (culture R4C5) was 1.65 times the top yield obtained from culture R1C1. The average yields of tops obtained from Knop's solution and from Tottingham's best solution, both having the same total concentration as culture R1C1 (0.1 atm.), were 1.35 and 1.62, respectively. Thus the three-salt solution with proportions giving the highest yield of wheat tops, shows an improvement of 22 per cent over Knop's solution, while Tottingham's best solution shows an improvement over Knop's solution of 20 per cent, all for a total concentration of 0.1 atmosphere.

For the optimal series the highest average yield of tops (culture R5C2) was 1.39 relative to the yield from culture R1C1. The corresponding yields obtained from Knop's solution and from Tottingham's best solution for tops were 1.09 and 1.27, respectively, relative to the same control. For this total concentration (1.75 atm.) the best salt proportions of the three-salt solution produced an average dry weight of wheat tops 28 per cent higher than the yield obtained from Knop's solution with the same total concentration, while Tottingham's best solution for wheat tops produced a corresponding average yield 17 per cent above that obtained from Knop's solution.

For the supra-optimal series (4.0 atm.), the highest average dry weight of tops (culture R4C3) was 1.38 relative to the yield obtained from culture R1C1. Knop's solution and Tottingham's best solution for tops, with the same total concentration, yielded corresponding dry weights of 1.05 and 1.23, respectively. The best salt proportions of the 3-salt solution with this total concentration (4.0 atm.) showed an improvement of 31 per cent over Knop's solution, while Tottingham's best solution showed a corresponding improvement of 17 per cent.

In order to compare 3-salt solution R5C2, which produced the highest yield of wheat tops in the optimal series of the present studies, with Tottingham's best solution for wheat tops and with Knop's solution, all *with the same total concentration as was employed by Tottingham* (2.50 atm.) a series of three cultures of each of these three solutions, all having a total concentration of 2.50 atmospheres, was carried out during the 23-day period from February 25 to March 19, 1915. The average dry weight of tops produced by three-salt solution R5C2, relative to the average yield produced by Knop's solution was 1.22, and the corresponding average yield from Tottingham's best solution for tops was 1.07. Therefore, with a total concentration of 2.50 atmospheres, Tottingham's best solution produced a yield of tops 7 per cent higher than that obtained from Knop's solution, while the three-salt solution with the best salt proportions for wheat tops produced a corresponding yield 22 per cent higher than that produced by Knop's medium. The data for all these comparisons are presented in table XIII, which gives the volume-molecular partial concentrations of the solutions and the average dry weights of tops, relative to the yields obtained from the same total concentration of Knop's solution taken as unity. The actual values obtained with Knop's solution are given in parenthesis below the assumed relative values of unity.

TABLE XIII

Comparison of yields of wheat tops obtained from various total concentrations of Knop's solution, Tottingham's solution No. T3R1C4, and 3-salt solution No. R5C2.

TOTAL CONCENTRATION	NAME OF SOLUTION	PARTIAL MOLECULAR CONCENTRATIONS				YIELD OF TOPS RELATIVE TO THAT FROM KNOP'S SOLUTION
		KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	
atm.		*g.-mol. per l.*	*g.-mol. per l.*	*g.-mol. per l.*	*g.-mol. per l.*	
0.10	Knop's solution	0.00028	0.00022	0.00070	0.00024	1.00
	Tottingham's solution					(0.3527)
	No. T3R1C4	0.00062	0.00019	0.00058	0.00046	1.20
	3-salt solution No. R5C2		0.00082	0.00074	0.00028	1.22
1.75	Knop's solution	0.0059	0.0044	0.0145	0.0050	1.00
	Tottingham's solution					(0.4485)
	No. T3R1C4	0.0034	0.0108	0.0101	0.0081	1.17
	3-salt solution No. R5C2		0.0180	0.0052	0.0150	1.28
2.50	Knop's solution	0.0083	0.0063	0.0210	0.0070	1.00
	Tottingham's solution					(0.6807)
	No. T3R1C4	0.0049	0.0156	0.0144	0.0116	1.07
	3-salt solution No. R5C2		0.0257	0.0074	0.0214	1.22
4.00	Knop's solution	0.0198	0.0147	0.0488	0.0166	1.00
	Tottingham's solution					(0.3774)
	No. T3R1C4	0.0114	0.0354	0.0336	0.0278	1.17
	3-salt solution No. R5C2		0.0368	0.0198	0.0428	1.31

The comparative efficiencies of the 3-salt solutions R5C2 and R3C3 of the optimal series, with the best and second best salt proportions for dry weight yields of wheat tops, respectively, and of various other nutrient solutions commonly employed by workers in physiology, were determined by a special experiment. This experiment consisted in a duplicate series of cultures, all having a total concentration of 1.75 atmospheres, which was conducted during the 24-day period from December 17, 1914, to January 10, 1915.

The following eleven solutions devised by earlier writers were included in this comparison. The formulas are given in the list below, in terms of gram-molecules per liter (m). A trace of ferric phosphate was added to the solutions for which iron is not mentioned in the formula. As thus made up these solutions all agree with the two 3-salt solutions with which they are compared in having a total concentration of 1.75 atmospheres.

Birner and Lucanus'[31] *solution:* $Ca(NO_3)_2$, 0.0133 m; KH_2PO_4, 0.0108 m; $MgSO_4$, 0.0061 m; $Fe_3(PO_4)_2$, 0.0043 m.

Crone's[32] *solution:* KNO_3, 0.0266 m; $Ca_3(PO_4)_2$, 0.0022 m; $Fe_3(PO_4)_2$, 0.0019 m; $MgSO_4$, 0.0056 m; $CaSO_4$, 0.0049 m.

Detmer's[33] *solution:* $Ca(NO_3)_2$, 0.0149 m; KH_2PO_4, 0.0035 m; $MgSO_4$, 0.0051 m; KCl, 0.0083 m.

Hartwell,[34] *Wheeler and Pember's solution:* $Ca(NO_3)_2$, 0.0136 m; $CaH_4(PO_4)_2$, 0.0027 m; $MgSO_4$, 0.0077 m; KCl, 0.0075 m.

Knop's [1862] *solution:* KNO_3, 0.0059 m; KH_2PO_4, 0.0044 m; $Ca(NO_3)_2$, 0.0145 m; $MgSO_4$, 0.0050 m.

Pfeffer's[35] *solution:* $Ca(NO_3)_2$, 0.0136 m; KNO_3, 0.0056 m; KH_2PO_4, 0.0041 m; $MgSO_4$, 0.0046 m; KCl, 0.0037 m.

Sachs' [1860] *solution:* KNO_3, 0.0155 m; $Ca_3(PO_4)_2$, 0.0025 m; $MgSO_4$, 0.0065 m; $CaSO_4$, 0.0075 m; NaCl, 0.0067 m.

Schimper's[36] *solution:* $Ca(NO_3)_2$, 0.0118 m; KNO_3, 0.0048 m; KH_2PO_4, 0.0028 m; $MgSO_4$, 0.0040 m; NaCl, 0.0083 m.

Schreiner and Skinner's solution: $NaNO_3$, 0.0278 m; $CaH_4(PO_4)_2$, 0.00066 m; K_2SO_4, 0.0030 m.

Tollens'[37] *solution:* $Ca(NO_3)_2$, 0.0128 m; KNO_3, 0.0052 m; KH_2PO_4, 0.0038 m; $MgSO_4$, 0.0049 m; NaCl, 0.0054 m.

Tottingham's [1914] *solution:* KNO_3, 0.0034 m; KH_2PO_4, 0.0108 m; $Ca(NO_3)_2$, 0.0101 m; $MgSO_4$, 0.0081 m.

Table XIV presents the results of this comparative study. The notation of this table is self-explanatory. The solutions are arranged in the order of the total dry weight produced.

From table XIV it is clear that the two 3-salt solutions here tested (R5C2 and R3C3, total concentration 1.75 atm.) proved to be as good as or better than any of the other solutions mentioned, when employed with this total concentration. These two 3-salt solutions may be considered as practically equal in the production of top yields and of total dry weight, but it appears from the data just given that solution R5C2 is considerably better suited to the production of root yields than is solution R3C3. This latter indication is not clearly borne out by the other tests of these solutions, however. From table IV it is seen that solution R3C3 (1.75 atm.) gave in one instance (series A) a somewhat higher yield of roots than did solution R5C2 in the same test. On the other hand, in another test (series B) the relation between these two solutions, as to root yield, is reversed, and is similar to that brought out in table XIV. The averages of series A and B (table IV) indicate that these two solutions are practically equal in the production of root yields. These same averages indicate that solution R5C2 gives top yields a little higher than those obtained from solution R3C3. On the whole, it seems advisable to employ solution R5C2 (1.75 atm.) as the best solution. for wheat, resulting from the present study.

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[31] Birner, H., and Lucanus, B., Wasserkulturversuche mit Hafer in der Agric.-Chem. Versuchsstation zu Regenwalde. Landw. Versuchsst. **8:** 128–177. 1866. Note that this solution contains the same main salts as do the 3-salt solutions that are the subject of the present study, and that its proportions are nearly the same as those of 3-salt solution R3C6. The iron of Birner and Lucanus' solution is supplied as *ferrous* phosphate, however.

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[34] Hartwell, B. L., Wheeler, H. J., and Pember, F. R., The effect of the addition of sodium to deficient amounts of potassium upon the growth of plants in both water and sand cultures. Ann. Rep. Rhode Island Agric. Exp. Sta. **20:** 299–357. 1907.

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TABLE XIV

Relative dry weights of tops and of roots of wheat grown 24 days (Dec. 17, 1914, to Jan. 10, 1915) in various nutrient solutions, all having a total osmotic concentration of 1.75 atmospheres.

AUTHOR OF SOLUTION	RELATIVE YIELD OF TOPS	RELATIVE YIELD OF ROOTS	RELATIVE TOTAL DRY WEIGHT
Sachs	1.00* (0.362)	1.00 (0.134)	1.00 (0.497)
Schimper	0.95	1.14	1.00
Detmer	0.95	1.22	1.03
Tollens	1.28	1.10	1.23
Schreiner and Skinner	1.42	0.83	1.26
Hartwell, Wheeler and Pember	1.55	0.96	1.39
Pfeffer	1.52	1.13	1.41
Knop	1.60	1.07	1.45
Tottingham	1.77	1.13	1.60
Crone	1.95	1.05	1.70
Birner and Lucanus	1.93	1.17	1.72
Shive, R3C3	1.93	1.16	1.73
Shive, R5C2	1.93	1.22	1.74

* The data are each expressed in terms of the corresponding value obtained from Sachs' solution, considered as unity. The actual values for Sachs' solution in grams, are given in parenthesis.

The only instance in which a yield of 3-salt solution R5C2 (1.75 atm.) is surpassed by one of the other solutions with 1.75 atmospheres of total concentration occurs with Crone's solution, which gave a slightly higher yield of tops than did solution R5C2 (1.95 as compared to 1.93). But

Crone's solution here gave a much lower yield of roots and a slightly lower total yield than did solution R5C2.

Two instances are recorded in table XIV showing other solutions as giving yields equal to the corresponding one from solution R5C2. (1) Birner and Lucanus' solution gave as high a top yield, but it fell far below solution R5C2 in root yield and slightly below it in total yield. Solution R3C6 of the present study, which has the same main salts in nearly the same proportions as has Birner and Lucanus' solution, appears to be markedly inferior to solution R5C2 in the production of both tops and roots, according to the data of table IV (page 349). But the Birner and Lucanus solution contains ferrous phosphate ($Fe_3(PO_4)_2$) instead of the ferric salt employed with the 3-salt solutions of the present paper. (2) Detmer's solution equals solution R5C2 in root yield but is obviously very poor by the criterion of top yield and by that of total dry weight.

It should be emphasized in this connection that the conclusions here reached, as to the relative values of these various solutions, are to be taken as established only for the total concentration here employed (1.75 atm.), and for the kind of plant here used (winter wheat), during the first four weeks of its development from the seed. Whether some of the other solutions listed in table XIV may or may not surpass 3-salt solution R5C2, when used with other total concentrations, or with other plants, or other developmental stages of the same plant, or even under other climatic conditions cannot now be stated.

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PHYSIOLOGICAL TEMPERATURE INDICES FOR THE STUDY OF PLANT GROWTH IN RELATION TO CLIMATIC CONDITIONS[1]

BURTON EDWARD LIVINGSTON

ABSTRACT[2]

This publication deals with the derivation and use of a series of physiological indices of temperature efficiency for plant growth. These indices are derived from the data obtained by Lehenbauer in his study of the relation of temperature to the rate of elongation in seedling maize shoots. The system of indices thus obtained differs from the other systems of such indices so far proposed in three main particulars: (1) It is based on actual physiological experimentation. (2) It takes account of the general principle of temperature minima, optima and maxima. (3) It shows a much greater rate of increase for the index value, with rising temperature between 36° and 90°F. (2°–32°C.), than does either of the other systems. On the whole, the physiological system is to be regarded as preferable to the differential or exponential system.

By the use of these new indices, *physiological summation indices* of temperature efficiency for plant growth during the period of the average frostless season have been obtained for a large number of stations in the United States, and these have been charted so as to produce a new temperature-zone map of the country. This map shows isoclimatic lines having much the same general directions as those exhibited by most other temperature charts of the United States, but the range of the various summation indices is much greater in the case of the new chart than in that of the earlier ones based on summations. Thus, the remainder and exponential charts of Livingston and Livingston show that the seasonal temperature efficiency for Key West, Fla., is about 5.4 times as great as for Eastport, Me., while the physiological chart shows this ratio as 14.8. This difference is of course correlated with the relatively very rapid rise in index values, as related to increase in temperature, which has been emphasized in the preceding paragraph.

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[2] The manuscript of this paper was received January 1, 1916. This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 1, no. 8, March, 1916.

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While no one of the three systems of temperature efficiency indices above considered is to be regarded as probably true for any single plant form throughout its development, or for the various plant forms in general, yet the probability appears to be entirely in favor of the physiological system of indices as most nearly approaching a true expression of the relation of temperature to plant growth in general.

INTRODUCTION

Temperature is known to be one of the most influential of all the environmental conditions that take part in the control of plant growth, and yet no satisfactory expression of the relation here encountered has yet been suggested. Indeed, no suggestion so far made in this connection is even theoretically quite plausible. In an attempt to place ecological, agricultural and forestal science upon a more quantitative and hence more satisfactory and secure basis, the temperature relation of plants should therefore be one of the first to receive attention. The present paper deals with what appears to be an advance in the interpretation of climatological temperature data with regard to plant growth.

It is obvious that the temperature readings or temperature means so far generally employed in climatology can not, in themselves, be of great value as quantitative indices of the influence of the corresponding temperatures upon plant growth, though of course they are not without some meaning of a qualitative sort. About all that can be derived from a comparison of ordinary temperature data with plant growth rates is the generalization that temperatures may be either too low or too high for the most rapid development of any particular plant and of plant life in general For the comparison of the temperature conditions of different geographical localities, or of the same locality for different seasons, with reference to possible plant development, it has long been clear that some function of the temperature readings other than those generally used in climatology must be employed. Phenological writers[3] have suggested numerous ways of interpreting the temperature readings for this purpose, but, while some of these methods appear to have given satisfactory results for the particular regions for which they have been tried, none of them rests upon sound physiological reasoning and they cannot be expected to be of great general value. What appears to be requisite in this general connection is some kind of temperature indices which shall express the temperature values, not in ordinary thermometric terms but in terms of the possible *effectiveness* of the observed temperatures in determining the rate of plant growth.

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[3] An excellent and very full résumé of the numerous papers dealing with the temperature relations of plants, up to the time of its publication, is given by Abbe:—Abbe, C., A first report on the relations between climates and crops. U. S. Weather Bureau Bull. 36. Washington, 1905.

Two general classes of such temperature efficiency indices for plant growth have been proposed, remainder indices and exponential indices. The first of these are derived by substracting a constant quantity from each of the temperature data to be employed. Thus 39, 42, etc., may be subtracted from each daily mean temperature (Fahrenheit) throughout the season in question, a new series of numbers being thus obtained, which have been supposed to represent the relative growth rates of plants for these respective days. It is seen at once that this method of subtraction consists in fixing a daily temperature mean at which the growth rate is regarded as unity (40°, 43°, etc., Fahrenheit) and in supposing that growth rates for higher means are proportional to the corresponding differences obtained by the respective subtractions. Thus, if plants are supposed to grow at a rate of unity on a day with a mean temperature of 40°F. they would be supposed to grow with rates of 2, 3, etc., on days having means of 41°, 42°, etc., the constant difference being 39 in this case. Of course any thermometer scale may be employed, the constant difference being chosen accordingly. This remainder method for deriving indices of temperature efficiency for plant growth, from climatological temperature data, has been largely employed by phenological students, and a considerable literature rests upon it.

The exponential method for deriving these temperature efficiency indices was devised a few years ago by Livingston and Livingston.[1] It is based on the supposition that plant growth-rates follow the chemical principle of van't Hoff and Arrhenius, which states that chemical reaction velocities about double with each increase in temperature of 10°C., or 18°F. On this basis, the authors just mentioned derived their efficiency indices for growth from the equation,

$$u = 2^{\frac{t-40}{18}}$$

where u is the index to be derived and t is the temperature value, on the Fahrenheit scale. The index is thus seen to be an exponential or logarithmic function of the temperature itself as defined with reference to the thermometer scale.

In the study carried out by Livingston and Livingston the efficiency index corresponding to a temperature of 40°F. was taken as unity, so that the index for 58° became 2, that for 76° became 4, etc. According to this series of indices the growth rate of plants is supposed to increase with higher temperatures (but much less rapidly than according to the remainder series described above), as is shown by table I. This table presents samples of the two kinds of temperature efficiency indices thus far considered, for the temperatures from 40° to 45° and from 95° to 100°F.

Both methods for deriving these temperature efficiency indices agree in that the index values increase as the values representing temperature itself become larger, the relation between the two increasing series of numbers being a linear one in the first case and an exponential one in the second, but for the temperature range encountered in natural climates the second series also somewhat nearly approaches being linear.

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[1] **Livingston, B. E., and Livingston, Grace J.,** Temperature coefficients in plant geography and climatology. Bot. Gaz. **56**: 349–375. 1913.

The two lower graphs of figure 1 (I and II) represent the rates of increase of these two kinds of efficiency indices, as the temperature itself rises. Here the abscissas represent temperature, in degrees centigrade, and the ordinates represent the respective index values. It will be noted that both of these graphs coincide for the temperature 4.5°C. (40°F.), so that the slopes of the two are directly comparable. The numbers at the left of the figure refer to the ordinate values expressed in terms of the ordinate value for 4.5°C.

TABLE I

Remainder and exponential indices supposed to represent temperature efficiency for plant growth, for temperatures from 40° to 45° and from 95° to 100°F., considering the index for 40° as unity in both series

TEMPERATURE, FAHRENHEIT	REMAINDER INDEX	EXPONENTIAL INDEX
40	1	1.00
41	2	1.04
42	3	1.08
43	4	1.12
44	5	1.17
45	6	1.21
95	56	8.31
96	57	8.64
97	58	8.98
98	59	9.33
99	60	9.70
100	61	10.08

Graph I represents the remainder series of indices, and is seen to be linear. Graph II represents the exponential series, derived as just explained, and it is seen to be a logarithmic curve, concave upward. For the temperature range met with in natural climates graph II lies entirely below graph I, although the two intersect at a much higher temperature than needs to be here considered. The rate of increase of the first series of indices is thus seen to be much more rapid than is that of the second, within the climatic temperature range.

The authors last mentioned have called attention to the fact that the exponential series has a much more definite basis in physiological experiment than has the other, and that neither series can be supposed to represent temperature efficiencies for plant growth, even approximately, excepting for a limited temperature range. They do not attempt to state what may be the range of temperatures within which either series may be regarded as valuable, but it has long been clear that the highest temperatures experienced by plants in nature do not generally allow nearly as rapid growth as do somewhat lower temperatures, even though the other environmental conditions are at their optimum. This follows from the general principle of minimum, optimum and maximum temperatures for growth, as established by Sachs and supported by all later experimentation; as the temperature rises through the climatic range the growth rates of all organisms sooner or later cease to increase and begin to decrease.

It is this last consideration that throws the main *a priori* doubt upon the permanent usefulness of either the remainder or exponential indices as above described, no matter how satisfactory these may have appeared to be in some phenological tests, for temperatures are frequently encountered in nature that are beyond the upper limit below which these indices may be regarded as approximately true. It is obvious that the index values, in order to express the corresponding relative growth rates, must not continue to increase in magnitude as the temperature itself rises beyond this critical point, the so-called optimum for growth. In other words, the indices should not form a continuously increasing series of magnitudes, whether linear or exponential, but should attain a maximum value and then decrease again to zero.

DERIVATION OF PHYSIOLOGICAL TEMPERATURE INDICES

To derive such indices as are required it is thus necessary to refer to physiological experiment, from which alone can be obtained the rate of increase of the series, the position of the maximum value in terms of temperature itself, and the rate of decrease after the maximum is passed. It has been the lack of adequate physiological studies upon the relation of temperature to plant growth that has prevented any previous attempt in this dir ction, and the possibility of the present study is due entirely to the recent publication of Lehenbauer's[5] extensive researches in this field.

The last-named author determined the average hourly rates of elongation of the shoots of seedling maize plants (10–12 cm. high) when exposed, for periods of from 3 to 21 or more hours, to maintained temperatures of from 12° to 43°C. The plants were exposed in a chamber with moderate air circulation, the air humidity being maintained at approximately 95 per cent. Light conditions fluctuated, with the daily rhythm, between very weak, diffuse day-light and darkness. The increments of elongation were measured at 3-hourly intervals for the low and for the high temperatures, and at hourly intervals for the rest.

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[5] Lehenbauer, P. A., Growth of maize seedlings in relation to temperature. Physiol. Res. 1: 247–288. 1914.

Lehenbauer's graphs of the relation between maintained temperature, period of exposure, and average hourly rate of elongation of his maize shoots, show clearly the existence of a mininum, optimum and maximum temperature for this kind of growth, but the minima and maxima were not actually attained in his series. The optimum temperature varies with the period of exposure for which the average hourly growth-rate was determined. For a 3-hour period the optimum temperature was 29°C., for a 6-hour period it was 30°C., for 9-hour, 12-hour and 21-hour periods it was 32°. In other respects Lehenbauer's graphs for different exposure periods do not differ much in form; they are all concave upward in the regions of low and of high temperatures and convex upward in the vicinity of the optimum temperature. The graphs for the longer periods of exposure are smoother than those for the shorter ones, as might be expected.

Lehenbauer's graph for a 12-hour exposure period was taken as basis for the present study. This graph (with his table VII) shows that the average hourly rate of elongation of his maize shoots, exposed to maintained temperatures for 12 hours, was 0.09 mm. for 12°C., 1.11 mm. for 32°C., and 0.06 mm. for 43°C. His graph is here reproduced as the broken line of figure 1, the small circles showing the temperatures actually tested. In order to eliminate most of the irregularities, this graph is here smoothed by means of a spline, and the smoothed form is presented by the broad, full line of the figure. The smoothed graph is extended at its ends, by extrapolation, so as to intersect the horizontal axis at 2° and at 48°C. This treatment thus supposes that the last-named temperatures are, respectively, the minimum and maximum for growth under the given conditions. This supposition cannot be far from the truth, and, as will shortly appear, a considerable error in these regions of the graph would be without important effect upon the results obtained by the use of these indices in climatological interpretation.

The next step in the derivation of the indices was to measure the ordinates of the smoothed graph for each degree of temperature considered, on the centigrade and on the Fahrenheit scale, the numbers thus obtained representing average hourly rates of elongation, in hundredths of a millimeter. Then all the numbers of each of these two series were divided by the value for 4.5°C. (40°F.), thus giving the physiological indices sought. Each index expresses the average hourly growth-rate for its corresponding temperature, in terms of the growth-rate for 4.5°C. considered as unity, as in the cases of the remainder and exponential indices described above. Thus all three graphs of figure 1 coincide for the temperature 4.5°C. (40°F.). Table II presents the two series of physiological indices, one for employment when temperatures are given in centigrade degrees, the other for use with Fahrenheit temperatures.

From table II it is seen that the growth-rate for 32°C. (89.6°F.) is 122.3 times as great as that for 4.5°C. (40°F.). It also appears that the growth

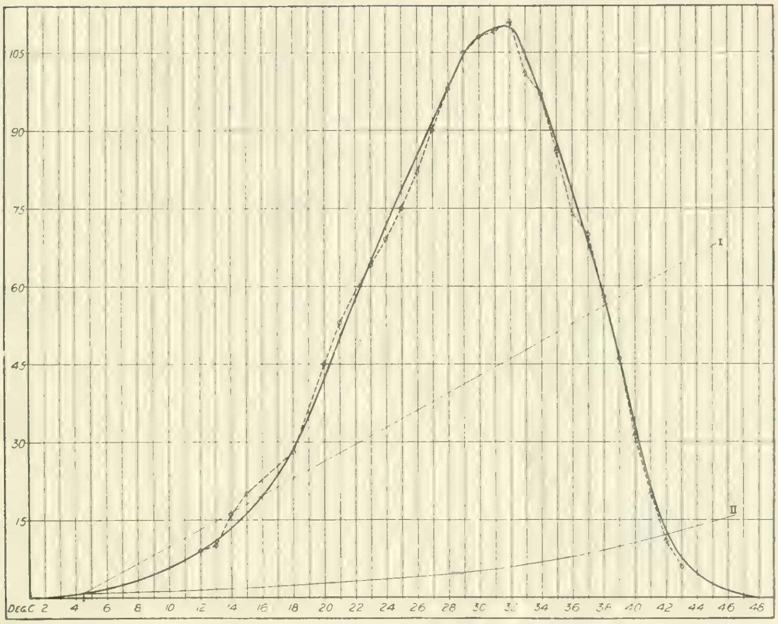

FIG. 1. Graphs showing increase in value of index of temperature efficiency for plant growth (ordinates) with rise in temperature itself (abscissas), for the three systems of indices. Graph I represents the remainder system, graph II the exponential one. The broken line is Lehenbauer's graph of the relation of temperature to the rate of elongation of the shoots of maize seedlings. The smoothed graph corresponding to the latter represents the physiological system of indices. All graphs pass through unity at 4.5°C.

TABLE II

Physiological indices of temperature efficiency for plant growth, based on Lehenbauer's measurements for 12-hour exposures with maize seedlings

CENTIGRADE SCALE		FAHRENHEIT SCALE			
Degrees Centigrade	Index	Degrees Fahrenheit	Index	Degrees Fahrenheit	Index
3	0.333	36	0.111	81	103.000
4	0.667	37	0.222	82	106.889
4.5	1.000	38	0.342	83	110.778
5	1.333	39	0.667	84	115.000
6	1.889	40	1.000	85	118.111
7	2.778	41	1.333	86	120.000
8	3.667	42	1.667	87	121.222
9	4.889	43	2.000	88	122.000
10	6.333	44	2.344	89	122.333
11	8.000	45	3.000	90	121.667
12	9.889	46	3.500	91	117.667
13	12.111	47	4.000	92	113.444
14	14.778	48	4.778	93	108.333
15	17.778	49	5.500	94	103.333
16	21.556	50	6.333	95	96.000
17	26.000	51	7.111	96	91.444
18	31.333	52	8.167	97	85.000
19	38.000	53	9.222	98	79.444
20	46.000	54	10.333	99	73.111
21	54.778	55	11.667	100	66.667
22	63.444	56	12.778	101	60.000
23	71.111	57	14.444	102	52.667
24	79.111	58	16.111	103	44.444
25	86.556	59	17.778	104	36.000
26	94.000	60	19.883	105	28.667
27	101.222	61	22.000	106	21.889
28	108.444	62	24.333	107	16.778
29	115.778	63	27.111	108	12.556
30	120.000	64	30.000	109	9.444
31	121.889	65	33.333	110	7.000
32	122.333	66	37.222	111	5.222
33	116.111	67	41.333	112	3.778
34	107.333	68	46.000	113	2.778
35	96.000	69	50.833	114	2.000
36	86.556	70	56.000	115	1.444
37	75.667	71	60.333	116	1.000
38	64.333	72	65.333	117	0.500
39	50.667	73	69.000	118	0.111
40	36.000	74	73.667		
41	23.333	75	78.111		
42	14.000	76	82.333		
43	8.333	77	86.556		
44	4.889	78	90.667		
45	2.778	79	95.000		
46	1.667	80	98.667		
47	0.667				

rate for 116°F. is the same as that for 40°F., and it is clear that, in general, each index value between zero and 122.3 corresponds to two temperature values, one on the ascending portion of the main graph of figure 1 and the other on the descending portion. All temperatures above 48°C. (118.4°F.) and below 2°C. (35.6°F.) have indices of zero. This series of indices takes account of the important fact that an exposure to a high temperature may produce the same growth-rate as does an exposure to a low temperature.

Whenever some of the temperatures dealt with in ecological or physiological studies are above 32°C. (89.6°F.) this system of physiological indices for growth must give markedly different results from those obtained with the remainder or exponential system. That natural shade temperatures above this critical point are infrequent in regions where ecology has been most studied, is apparently the reason why these two indefinitely increasing series have appeared so satisfactory in practical application, as in the cases presented by Livingston and Livingston. But for a general system of temperature interpretation with respect to plant growth the physiological indices are surely to be preferred to either of the other kinds.

Aside from the fact that the physiological system of indices takes account of the low growth-rates occurring with high temperatures, it appears preferable to either of the other systems because the slope of the series (the rate of increase in index value with rise in temperature) is based upon physiological experimentation over a broad range of temperatures. The van't Hoff-Arrhenius principle, upon which is based the exponential series, appears to hold for the elongation of young maize shoots only for a temperature range from about 20° to about 30°C. (Lehenbauer), and the physiological system is approximately true for all temperatures from 12° to 43°C., at least for the conditions of Lehenbauer's experiments.

As is shown by figure 1, the rate of increase in index value with rise of temperature, between the minimum and optimum for growth is much more rapid in the case of the physiological series than in that of either of the other series. The range of temperature thus indicated (from 2°C. or 35.6°F. to about 32°C. or 89.6°F.) is the range usually encountered in nature during the frostless season, at least in temperate climates, and most of the plant growth of the world is probably accomplished with temperatures lying within this range. Practically, this very rapid rise in the index values constitutes the most important difference between the physiological series and the other two.

While it is quite apparent that the system of physiological indices here described is far superior, in several respects, to the other systems heretofore suggested, it is equally clear that these indices are to be regarded as only a first approximation and that much more physiological study will be required before they may be taken as generally applicable. In the first place, they are based upon tests of only a single plant species, maize, and there are probably other plants (perhaps even other varieties of the same species) for which they are not even approximately true. Second, these indices are derived from the growth of seedlings, and no doubt other phases of growth in the same plant may exhibit other relations between temperature and the rate of shoot elongation. Third, these indices refer to rates of shoot elongation, and there are many other processes involved in plant growth, which may require other indices for their proper interpretation in terms of temperature efficiency. Fourth, they apply strictly only under the moisture, light and chemical conditions that prevailed in Lehenbauer's experiments; with more light or with a different light mixture, with different humidity conditions, or with different moisture or chemical surroundings about the roots, these same plants, in the same seedling phase, may exhibit very different values of the temperature efficiency indices. Fifth, and finally, plants in nature are never subject to any temperature maintained for any considerable period of time, and these indices are derived from 12-hour exposures to maintained temperatures. As MacDougal[6] has well emphasized, the indices really needed for the ecological and physiological interpretation of temperature must take account of the varying temperatures that are almost always encountered in nature.

In the face of all these considerations it would be very rash indeed to suppose that the index series here brought forward may be found to apply in a wholly satisfactory way to all plants or even to plants in general. However, the amount of labor required to determine experimentally the index values for a single plant phase or form and for a single set or complex of non-thermal conditions is very great, and there is little hope for the early attainment of anything approaching the ideal set or sets of relations here suggested. It appears that the most promising way to project ecological science into such new fields as that here considered is to make what use is possible of present information, and to try to erect upon the beginnings of the past and present the more adequate appreciation of the quantitative relations of natural phenomena that forms the goal of all scientific effort. The argument so frequently put forward in favor of merely descriptive ecology and climatology, that we probably never shall be able to interpret plant phenomena quite satisfactorily in terms of environmental conditions, seems to the writer to be always wholly inapplicable; whether the ideal goal may or may not be ultimately attainable is of no present interest, and all that can logically be desired in the present and immediate future is to move forward a little in the direction of that goal. If apology seems to be required, this may be considered as the one here offered for the present bringing forward of such an ideally inadequate set of temperature efficiency indices as that just described.

It may be added, furthermore, that if such a system of indices is made to correspond to one developmental phase of one plant form, growing under one complex of non-thermal conditions and with one period of exposure to maintained temperature, the alterations or corrections necessary to render this system truthful within other conditional limitations may perhaps be more readily accomplished than would be the *de novo* derivation of the corrected system. It is frequently easier to set forth the form of a complicated object by showing how it differs from an incorrect diagram than it would be to draft the correct diagram without reference to the incorrect one.

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[6] MacDougal, D. T., The auxothermal integration of climatic complexes. Amer. Jour. Bot. 1: 186–193. 1914.

It may also be added that we have here the suggestion of one way by which the results of physiological studies such as that of Lehenbauer may be employed in lines of investigation that appear to be far removed from the studies themselves. Some large openings in the exceedingly leaky ship of physiological ecology might be stopped by just such studies as that of Lehenbauer, if it should be possible for a number of these to be carried out in a comparable manner. Other developmental phases, other plants and other complexes of non-thermal conditions will of course require attention.

CLIMATIC ZONES OF THE UNITED STATES DERIVED FROM THE PHYSIOLOGICAL INDICES OF TEMPERATURE EFFICIENCY FOR PLANT GROWTH

Interest in the climatological conditions of the United States, as related to plant distribution, has furnished the primary reason for the derivation of the physiological indices with which this paper deals, and the present section will be devoted to a study of the climatic zones of this country as derived from these indices. The general method employed by Livingston and Livingston for this sort of investigation will be followed. The average frostless season is taken as the duration factor for each station considered, and the normal daily mean temperatures for the various dates within this season, as calculated by Bigelow,[7] serve as basis for the intensity factor.

The procedure has been as follows: From table II is found the physiological index corresponding to the normal daily mean temperature[8] for each day of the period of the average frostless season, for each station. Then all these indices (there being as many as there are days in the period of the average frostless season for the station in question) are summed, giving what may be termed a *summation index* of seasonal temperature efficiency for plant growth.[9] The results obtained are set forth in column 3 of table III, which also includes, besides the station names, the respective lengths of the period of the average frostless season,[10] column 2.

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[7] Bigelow, F. H., The daily normal temperature and daily normal precipitation in the United States. U. S. Dept. Agric., Weather Bur. Bull. R. 1908.

[8] Fahrenheit temperature data are employed throughout this study, since the data published by the U. S. Weather Bureau are still given in these antiquated terms.

[9] These indices are simply magnitudes, and the term degree should never be applied to them.

[10] Derived from the Summary of the climatological data of the United States, by sections. Published by the U. S. Weather Bureau, about 1910, without date or imprint.

Summation indices of temperature efficiency for plant growth, for the period of the average frostless season, for 170 stations in the United States, based on the physiological system of indices of temperature efficiency derived from Lehenbauer's experiments.

STATION	LENGTH OF AVERAGE FROSTLESS SEASON	SUMMATION INDEX (PHYSIOLOGICAL SYSTEM, FAHRENHEIT SCALE)
	days	
Alabama		
Anniston	201	12326
Birmingham	231	15025
Mobile	279	17340
Montgomery	243	16511
Arizona		
Flagstaff	105	2562
Phoenix	283	20640
Arkansas		
Fort Smith	230	14168
Little Rock	237	14567
California		
Eureka	245	2388
Fresno	258	15007
Independence	222	11228
Los Angeles	334	8451
Red Bluff	264	14339
Sacramento	272	9884
San Francisco	319	4122
San Jose	294	7000
San Luis Obispo	260	4963
Colorado		
Denver	153	6271
Durango	121	4077
Grand Junction	183	9921
Pueblo	163	7604
Connecticut		
Hartford	165	6181
New Haven	180	6703
Florida		
Jacksonville	293	18791
Jupiter	318	24872
Key West	365	31063
Pensacola	285	18914
Tampa	335	21420
Georgia		
Atlanta	225	13019
Augusta	228	15134
Macon	238	14564
Savannah	263	16407
Georgia (cont.)		
Thomasville	257	17858
Idaho		
Boise	177	6716
Lewiston	202	8065
Pocatello	175	5893
Illinois		
Cairo	212	12122
Chicago	182	6892
La Salle	168	7975
Peoria	186	8466
Springfield	182	9464
Indiana		
Evansville	203	11879
Indianapolis	186	9441
Iowa		
Charles City	133	6630
Davenport	174	8464
Des Moines	171	8417
Dubuque	176	7865
Keokuk	197	9681
Sioux City	146	7548
Kansas		
Concordia	173	10206
Dodge	181	10308
Topeka	189	10546
Wichita	194	11563
Kentucky		
Lexington	187	10051
Louisville	196	11581
Louisiana		
New Orleans	310	19323
Shreveport	252	17023
Maine		
Eastport	167	2102
Portland	157	4362
Maryland		
Baltimore	213	10457
Washington, D. C.	197	10087

Summation indices—continued

STATION	LENGTH OF AVERAGE FROSTLESS SEASON	SUMMATION INDEX (PHYSIOLOGICAL SYSTEM, FAHRENHEIT SCALE)
	days	
Massachusetts		
Boston	185	6157
Nantucket	209	5032
Michigan		
Alpena	137	3277
Detroit	164	6622
Escanaba	140	3472
Grand Haven	167	5283
Grand Rapids	164	6687
Houghton	152	3147
Marquette	140	3019
Port Huron	155	4884
Sault Ste. Marie	138	2322
Minnesota		
Duluth	152	3299
Moorhead	132	4283
St. Paul	159	6230
Mississippi		
Meridian	230	14565
Vicksburg	252	16194
Missouri		
Columbia	179	10241
Hannibal	183	10189
Kansas City	196	10368
St. Louis	207	11868
Springfield	187	10031
Montana		
Havre	122	4036
Helena	144	3710
Kalispell	140	2827
Miles City	140	6253
Nebraska		
Lincoln	174	9062
North Platte	151	7192
Omaha	170	9087
Valentine	132	6393
Nevada		
Reno	138	4134
Winnemucca	131	5463
New Hampshire		
Concord	146	4724
New Jersey		
Atlantic City	207	7878
Cape May	186	8417
New Mexico		
Roswell	195	12448
Sante Fe	187	5350
New York		
Albany	177	6633
Binghamton	158	5309
Buffalo	173	5761
Canton	139	4713
Ithaca	160	5659
New York	210	8104
Oswego	175	5524
Rochester	171	5807
Syracuse	171	6022
North Carolina		
Asheville	176	7504
Charlotte	220	12554
Hatteras	256	13771
Raleigh	213	12329
Wilmington	233	13561
North Dakota		
Bismarck	129	4792
Devils Lake	121	3754
Williston	119	4508
Ohio		
Cincinnati	194	10725
Cleveland	198	7151
Columbus	184	8799
Sandusky	195	7766
Toledo	174	7508
Oklahoma		
Oklahoma	214	13098
Oregon		
Baker City	127	3116
Portland	245	4780
Roseburg	198	4465
Pennsylvania		
Erie	194	6741
Harrisburg	196	8367
Philadelphia	206	9397

Summation indices—continued

STATION	LENGTH OF AVERAGE FROSTLESS SEASON	SUMMATION INDEX (PHYSIOLOGICAL SYSTEM, FAHRENHEIT SCALE)
	days	
Pennsylvania (cont.)		
Pittsburgh	179	8659
Scranton	176	6463
Rhode Island		
Block Island	218	5447
Providence	190	7241
South Carolina		
Charleston	276	16874
Columbia	231	15140
South Dakota		
Huron	131	5604
Pierre	153	7566
Rapid City	143	5059
Yankton	154	7616
Tennessee		
Chattanooga	207	12395
Knoxville	208	10886
Memphis	224	14392
Nashville	207	12886
Texas		
Abilene	245	15937
Amarillo	199	10668
Corpus Christi	298	21392
El Paso	236	15043
Fort Worth	261	17652
Galveston	331	21163
Palestine	245	16468
San Antonio	276	19202
Taylor	254	18200
Utah		
Modena	130	4826
Salt Lake City	182	8416
Vermont		
Burlington	143	4341
Northfield	126	3348
Virginia		
Lynchburg	201	10631
Norfolk	230	12194
Richmond	215	12305
Wytheville	175	7313
Washington		
North Head	316	2693
Seattle	246	3692
Spokane	202	5059
Tatoosh Island	271	1947
Walla Walla	216	8378
West Virginia		
Elkins	145	5685
Parkersburg	179	9009
Wisconsin		
Green Bay	153	4942
La Crosse	163	6705
Madison	179	6434
Milwaukee	162	5261
Wyoming		
Cheyenne	119	3640
Lander	108	3548

The summation indices of temperature efficiency given in table III were plotted on a map, and isoclimatic lines were drawn in the usual way, dividing the area of the country into climatic zones. The resulting chart is reproduced as figure 2. From table III it appears that the summation index here considered ranges, in this list of stations, from a value of 1,947 (Tatoosh Island, Wash.), to one of 31,063 (Key West, Fla.), the latter value being about 16 times as great as the former. In other words, if these summation indices are indeed proportional to the temperature efficiency values of the various climates, the normal temperature conditions for Key West should be capable of producing about 16 times as much plant growth within the limits of the frostless season as are those for Tatoosh Island.

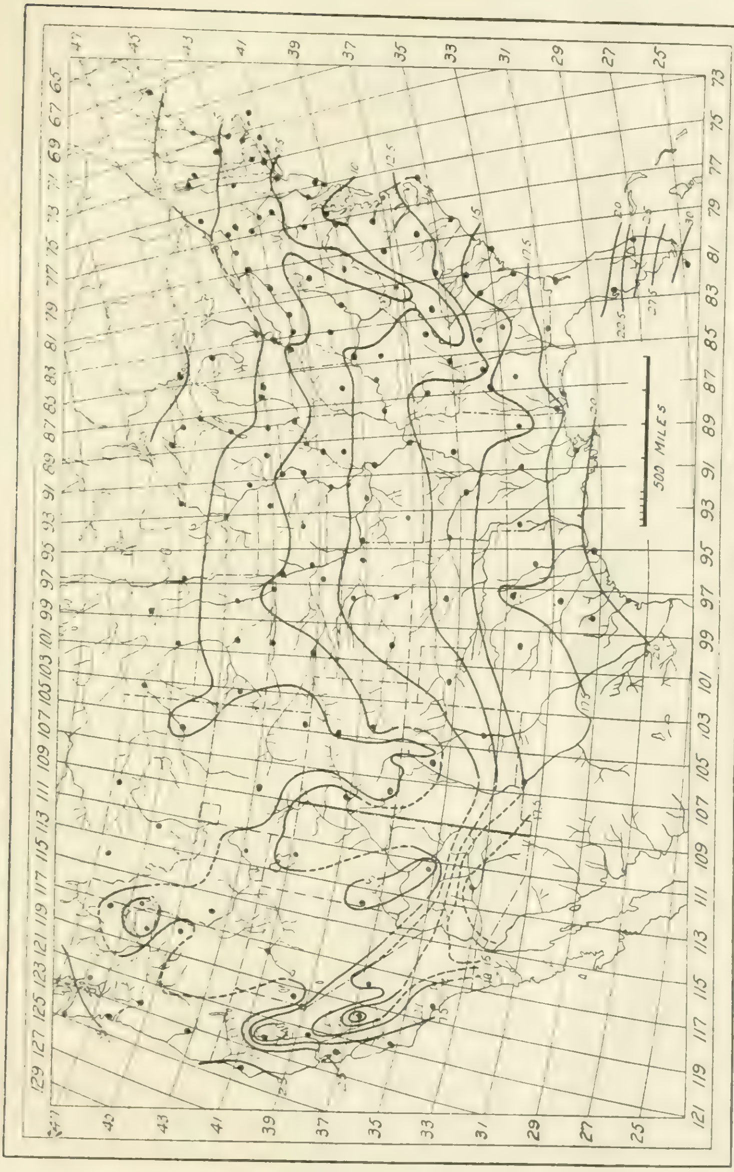

FIG. 2. Chart of the United States showing climatic zonation according to *physiological* summation indices of temperature efficiency for plant growth, for the period of the average frostless season. The numbers on the isoclimatic lines each represent thousands. Broken lines denote a very high degree of uncertainty.

The chart of figure 1 shows the country as divided into temperature efficiency zones having generally a west-east direction, like those of most other temperature charts, these zones being southwardly displaced in the regions of the two mountain systems and in that of the Pacific ocean. Other details may be observed, but do not require attention in the present state of our knowledge. It now becomes interesting to compare the chart of figure 2 with the two somewhat similarly derived charts of the Livingston and Livingston paper, which are here reproduced as figures 3 and 4. The chart of figure 3 was derived from the summation of remainder temperature efficiency indices (above 39°F.) and that of figure 4, from the summation of exponential indices (based on a supposed doubling of the growth-rate with each rise of 18°F.). Thus these three charts offer a comparison of the results obtained by using each of the three forms of efficiency indices in the same way.

The three charts are seen to be much alike in the general directions of their lines. The main differences to be noted are those that occur in the values represented by the numbers attached to the lines. The chart based on remainder indices (fig. 3) shows summation values that range from 2,397 (Flagstaff, Ariz.) and 2,423 (Burlington, Vt.) to 14,522 (Key West, Fla.), while the one based on exponential indices (fig. 4) shows values ranging from 245 (Flagstaff, Ariz.) and 276 (Sault Ste. Marie, Mich.) to 1,542 (Key West, Fla.). The two Livingston and Livingston charts therefore agree in showing summation indices that range from unity to about 6, while, as has been remarked, the new chart (fig. 2) shows a corresponding range of from unity to about 16. This difference furnishes a valuable criterion for use in determining whether the Livingston and Livingston charts (which are much alike in respect to this range) or the one based on physiological indices may more nearly express true temperature efficiency values for plant growth in the United States; obviously the temperature efficiency for Key West cannot be both 6 and 16 times as great as that for the stations showing the lowest values. Of course this question cannot be answered without much experimental work, involving the measurement of plant growth at various stations, but perhaps the need for and the importance of such measurement may be emphasized by the present study.

On general grounds it may be supposed, however, that the greater range more nearly approaches the truth, and some further evidence, of a kind not very definite but in the same direction, may be obtained from a study of the numerical values in two extreme cases. Since it is impossible to employ here the stations having the lowest and the highest summation indices and at the same time to employ the same two stations on all three charts, we may take as our two extremes in all cases Eastport, Me. and Key West, Fla. Table IV shows the various numerical data with which this discussion is to proceed. The table is self-explanatory.

An attempt to interpret the data of table IV, as far as this seems desirable

TABLE IV

Numerical data for the comparison of seasonal temperature efficiency for plant growth for the stations Eastport and Key West

	EASTPORT, ME.	KEY WEST, FLA.
Duration of period of average frostless season (D), *days*	167	365
Relative duration of period, that for Eastport taken as unity	1	2.2
Physiological summation index (A)	2102	31063
Relative physiological summation index, that for Eastport taken as unity	1	14.8
Mean daily physiological index for period $\left(\frac{A}{D}\right)$	12.6	85.1
Relative mean daily physiological index, that for Eastport taken as unity	1	6.8
Remainder summation index (B)	2606	14522
Relative remainder summation index, that for Eastport taken as unity	1	5.6
Mean daily differential index for period $\left(\frac{B}{D}\right)$	15.6	39.8
Relative mean daily differential index, that for Eastport taken as unity	1	2.6
Exponential summation index (C)	299	1542
Relative exponential summation index, that for Eastport taken as unity	1	5.2
Mean daily exponential index for period $\left(\frac{C}{D}\right)$	1.8	4.2
Relative mean daily exponential index, that for Eastport taken as unity	1	2.3

at present, will now be considered. In the first place, the summation index for the period of the average frostless season is to be looked upon as a product of two factors, one representing the duration of the temperature condition and the other, its mean daily intensity. The duration factor for Key West is 2.2 times as great as that for Eastport, so that if the average daily mean were the same for both stations (i. e., if there were no difference in the average intensity of temperature efficiency) the summation index for the southern station should be 2.2 times that for the northern. If the plants were to grow equally tor all days within the period of the average frostless season

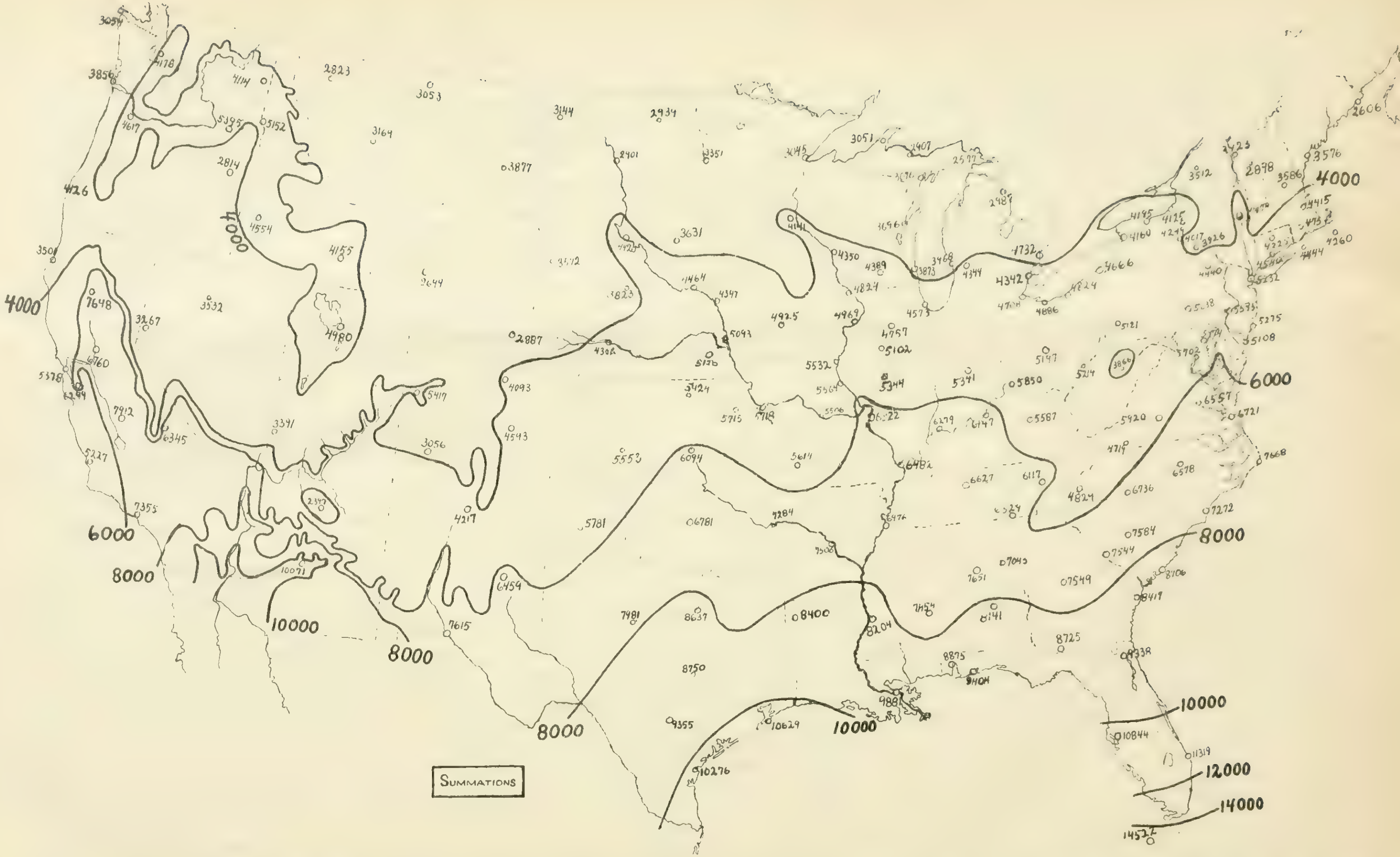

FIG. 3. Chart of the United States showing climatic zonation according to *remainder* summation indices of temperature efficiency for plant growth, for the period of the average frostless season. (Reproduced from Livingston and Livingston [1913])

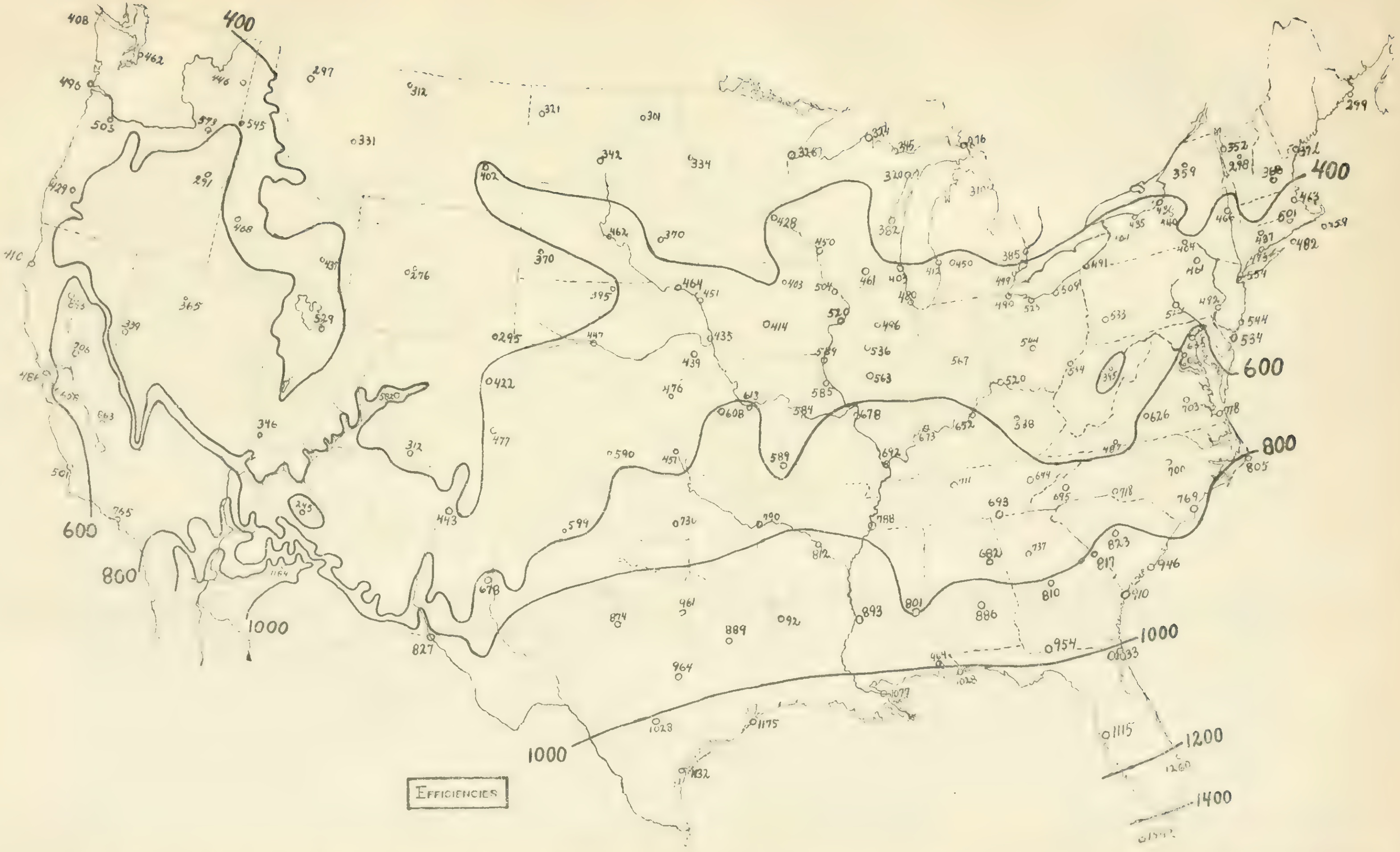

FIG. 4. Chart of the United States showing climatic zonation according to *exponential* summation indices of temperature efficiency for plant growth, for the period of the average frostless season. (Reproduced from Livingston and Livingston [1913])

for both stations they should grow 2.2 times as much in 365 days as in 167 days. This consideration applies, of course, to all three kinds of summation indices.

The second or intensity factor in the summation index is the mean daily temperature efficiency, the mean daily index, for the period. The relative values of these mean daily indices for Key West, derived by the three methods, taking the value for Eastport as unity in each case, are:

By the physiological system	6.8
By the remainder system	2.6
By the exponential system	2.3

It is clear that the remainder and exponential systems nearly agree in this regard, their average mean daily index being 2.45, while the mean daily index derived from the physiological system (6.8) is 2.8 times as great. Without experimentation other than that of Lehenbauer, it appears very probable that the difference between the average mean daily temperature efficiencies for plant growth, during the period of the average frostless season, at these two stations, is more nearly represented by the ratio 6.8 than by the ratio 2.5. At any rate this discussion shows one way by which the problem of temperature differences between different stations may be approached.

Either of the three systems of temperature indices here compared appears to give a rational zonation of the area of the United States. In all three cases the relative magnitudes of the summation indices for the period of the average frostless season are influenced not only by the normal daily temperature means but by the length of the period itself. For adequate interpretation of climate with reference to plant growth this is requisite, and in this particular all three systems of indices appear to be satisfactory on theoretical grounds.

The other theoretical consideration already emphasized (that the physiological system based on Lehenbauer's measurements is the only one of the three that takes account of low growth-rates with high temperatures) is not practically tested with the summation indices here calculated from the normal daily means; the only station with any daily means above 90°F. (the maximum of the Lehenbauer graph) encountered in this work is Phoenix, Ariz., so that the data for this station might alone be studied in this connection. But since the highest normal daily mean temperature for Phoenix is only 91°F., the daily efficiency indices for the very hottest days are not very markedly smaller than those for the days having daily normals of 90°F. When these indices come to be applied to hourly means instead of daily ones, the lower index values corresponding to the high temperatures will have a noticeable effect for the hottest season at many stations in the United States. Such more detailed application of the system of indices here proposed remains for the future, however.

So far as this kind of study has gone, the main argument in favor of the physiological indices for the evaluation of the temperature aspect of climate lies in the fact that these indices have been directly obtained from physiological data.

The tendency of biological writers generally to try to interpret life phenomena in terms of one, or at most very few, environmental conditions, may lead to misconception as to the aim of the kind of study here reported. Such studies are to be regarded as preparatory for work that may come later, and the results must always be taken in connection with the fact that many influential conditions are purposefully left out of account. Thus, this empirical study of temperature indices appears to exaggerate the importance of the temperature condition, and totally neglects the conditions of moisture, light, etc. To be more specific, it is obvious that the natural vegetation of Key West, for example, does not seem to bear out the supposition that the temperature climate of this area is 14.8 times as efficient in producing plant growth as is that of Eastport. The reason for this appears to lie in the fact that the vegetation of the Florida Keys is not controlled by temperature conditions alone, but by a complex of conditions (many of which are not even climatic in their nature). Temperature is only a single one of the many conditions making up this complex.

Actually to test the validity of such conclusions as are reached in the present paper, in regard to Key West and Eastport, for instance, it would be necessary to subject one group of similar plants to the temperature conditions of each of these two stations, at the same time arranging the experiment in such a way that moisture, light and soil conditions might be the same for both cultures. To accomplish this at present, in the open, is practically impossible, and even laboratory facilities for the actual study of environmental complexes have not as yet been established anywhere in the world. The need for such laboratory facilities has long been appreciated by a few biological workers, but the majority seem still to cling to the idea that organisms should always be studied under "natural" or "normal" conditions, interest having centered mainly in the simple description of living things and their dead remains, and of the behavior of organisms under the conditions that nature happens to afford at the time and place of carrying out the studies in question. Of course experimentation that does not take account of the quality, intensity and duration of all the effective environmental conditions involved can usually be little more than suggestive of the logical possibilities of the case. That it is difficult to obtain quantitative knowledge of all the environmental conditions influencing an organism is of course true, but this does not appear to be a logical reason for utterly neglecting most of the terms of the environmental complex.

What is most needed are adequate methods for measuring and integrating natural environmental conditions and for artifically controlling these conditions. As such methods are developed and as facilities for their use become available, the whole nature of biological science will be altered, but the first desideratum in this whole matter is a much broader appreciation of the needs here encountered, together with some conscious striving toward the attainment of these needs.

The suggestion here made, that real studies on the relations between external conditions and plant development are the only logical bases for a truly scientific ecology and agriculture, and that such studies of higher plants are not really possible without the elaborate facilities of specially constructed laboratories, was largely included in a plea for a climatic laboratory made by A. P. deCandolle as early as 1885, in his *Geographic Botanique Raisonée.* This idea seems never to have borne fruit. In 1891 Abbe [1905] repeated and endorsed the suggestion of deCandolle, but the utter lack of appreciation with which the arduous work of Abbe was received may be estimated from the mere fact that his summary lay unpublished for fourteen years. It was at length brought out—in apparently perfunctory form—only in 1905! Nevertheless, the dynamic aspect of biological enquiries must sooner or later attract general attention, and when this occurs the facilities for logical experimentation on environmental relations will probably be forthcoming. The present paper aims to be of service in the planning of adequate experimentation on these relations whenever such experimentation may become possible.

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A SINGLE INDEX TO REPRESENT BOTH MOISTURE AND TEMPERATURE CONDITIONS AS RELATED TO PLANTS[1]

BURTON EDWARD LIVINGSTON

ABSTRACT[2]

This paper suggests a method by which the moisture and temperature conditions of any locality, for any period, as they affect plants, may be expressed as a single numerical value, the index of moisture-temperature efficiency for plant growth. This index is the product of three factors, the index of rainfall, the reciprocal of the index of atmospheric evaporating power, and the physiological index of temperature efficiency.

The details remain to be worked out, but the analytical method here proposed appears already to have considerable value in studies dealing with the relation of these climatic conditions to plants. A chart representing the moisture-temperature conditions for 170 stations in the United States is derived by the employment of this method, and some features of its interpretation are set forth. As far as moisture and temperature conditions are concerned, it appears that peninsular Florida possesses the best climate for plant growth in general, while the least efficient climates of the country are those of the extreme north and of the arid regions.

INTRODUCTION

A newly aroused interest in the relation of climatic conditions to plant growth is demanding other units for the comparison of climates than those in common use by climatologists and meteorologists. This interest is related to the recent rapid advance in quantitative, or at least dynamic, physiology and physiological ecology, and also to the tendency of modern agriculture and forestry to become scientific in method and to consider plant growth from the view-point of the physiological processes involved. That the familiar monthly and annual averages or means of temperature. rainfall, humidity, etc., are of little value, as such, in quantitative comparison of climatic conditions with plant growth and with vegetational distribution,

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[1] Botanical contribution from the Johns Hopkins University, No. 51. This paper was presented, in very brief form, at the meeting of the Physiological Section of the Botanical Society of America, Columbus, December 29, 1915.

[2] The manuscript of this paper was received January 1, 1916. This abstract was preprinted, without change, from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 1, no. 9, April, 1916.

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has long been obvious enough. Yet students of climatology have been exceedingly loath to attempt any novel climatic observations or to undertake any new methods of interpreting the climatic and meteorological records that are already available, and it has appeared until recently as though the weather services of the world were too busy obtaining records and making forecasts to be able to undertake any serious study in the direction here considered. It must be added, however, that the more adaptable governmental services have already gone so far with studies of the relation of climate to crops that a new science seems to have arisen, *agricultural meteorology*. The Russian service is probably the leader in this work, and it has recently been taken up by the Canadian service. It seems highly probable that the special study of the relations of climate to plant growth will eventually become perhaps the most important part of the activities of climatologists.

Such investigation as is here suggested is of course a combination of applied plant physiology (or physiological ecology) and applied climatology, and the personnel of the new science, if science it may properly be called, will at first consist mainly of individuals who have come to this work through one of these two lines. Physiologists who are acquainted with climatology and climatologists who are acquainted with plant physiology may find here a field worthy of their best endeavors, both scientifically and practically.

While the official climatological services have largely refrained from entering into this work, there have been many individuals who have repeatedly called attention to the great need here suggested. Abbe has done much toward the inauguration of this sort of investigation in this country but his endeavors in this direction seem to have been checked at every turn by the conservatism of the U. S. Weather Bureau in this respect.[3] The progress that has been made is due largely to ecologists, agriculturists and students of the scientific aspects of forestry. The Bureau of Plant Industry of the U. S. Department of Agriculture has recently carried out many studies in this field and the U. S. Forest Service has also undertaken this kind of investigation. Outside of the Department of Agriculture various workers have become interested, and this new aspect of plant ecology is progressing rapidly in this country.

For many years I have been convinced that one of the chief desiderata of ecological science is a series of methods by which environmental conditions might be measured and compared,[4] as to their effectiveness in controlling plant processes. Ten years ago some of these conditions could be measured, but not in terms of their influence upon plants, and others were still quite beyond our reach, as far as quantitative comparisons are concerned. During the last decade a number of advances have been made and the accurate, quantitative description of environmental complexes is not now nearly so hopeless as it was in 1905. The most encouraging feature of the present status of physiological ecology, as compared with its status ten years ago, lies, however, in the fact that the number of workers interested in these fundamental problems of measurement is now much larger than it was then.

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[3] It is not without significance that Professor Abbe's book on this subject was held in manuscript for more than a decade before it was finally published. Abbe, C., First report on the relations between climates and crops. U. S. Dept. Agric., Weather Bur. Bull. 36. Washington, 1905.

[4] Livingston, B. E., Present problems of physiological plant ecology. Amer. Nat. **43**: 369–377. 1909.

Environmental conditions affecting plants may be classified in five groups, according to their nature, as follows:

(1) *Moisture conditions* are effective both above and below the soil surface (intensity of absorbed radiation, evaporating power of the air, soil moisture conditions). They are undoubtedly partly climatic, but there is nothing to be gained by attempting to define a group of conditions as exclusively climatic or nonclimatic.

(2) *Temperature conditions* are also influential upon both aerial and subterranean parts of the plant (air temperature, soil temperature).

(3) *Light conditions* are effective only above the soil, at least in general.

(4) *Chemical conditions* influence the plant both above and below the soil surface (chemistry of the atmosphere, of the soil air, and of the soil solution).

(5) *Mechanical conditions* are also influential both above and below the soil surface (wind pressure above the soil, animal activities both in air and soil, resistance offered by rocks, etc., mainly below the soil surface, etc.).

Moisture relations. Of the moisture relations we are now able to measure the evaporating power of the air[5] with considerable precision, and the moisture conditions of the soil promise to become measurable eventually.[6] In the absence of perfected methods for measuring soil moisture conditions as these affect plants, it becomes necessary to study the main one of the series of secondary conditions that, in turn, determine the soil moisture condition, namely rainfall. The soil moisture condition, for any given type of soil, may be considered as mainly controlled by rainfall and by atmospheric evaporating power, and Transeau[7] has employed the ratio of annual rainfall to annual evaporation from a standard surface as a measure of the moisture conditions in general, as these may affect vegetational distribution. This ratio appears to be a very valuable index of plant environment, as far as water relations are concerned.[8] It is surely the best index of environmental moisture conditions that is possible at present,[9] though I think it probable that the duration factor employed should be the length of the period of the average frostless season rather than that of the entire year.

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[5] The U. S. Weather Bureau has voiced serious objection to this term because the space of a vacuum would not be *air* in the true sense and would still exert what appears like an evaporating power. I employ the term as though such a vacuum might be called air, as it frequently would be if the matter ever came up for anything but purely academic discussion. Air here implies that portion of circumambient space about the plant which is not occupied by solid or liquid. There seems never to have been any attempt to define air according to its chemical content; it would still be air if it were largely carbon dioxide or hydrogen, etc., and it seems unadvisable to attempt a restriction of the terms air and atmosphere at this late day. For the rest, the expression, evaporating power of the air, has been in use among students of this power at least since 1906 (when I used it myself), and no suggestion of a better term has yet come to my notice. I shall suggest a more precise term at a later time, unless such an expression may arise from some other source, but the expression here in question will probably appeal to most students of this power as quite unobjectionable and it need not be dropped. Regarding this whole matter, see: Livingston, B. E., The relation of desert plants to soil moisture and to evaporation. Carnegie Inst. Wash. Pub. 50. Washington, 1906. *Idem*, Atmometry and the porous cup atmometer. Plant World **18**: 21–30, 51–74, 95–111, 143–149. 1915. *Idem*, Atmospheric influence on evaporation and its direct measurement. Monthly Weather Rev. **43**: 126–131. 1915. *Idem*, A modification of the Bellani porous plate atmometer. Science n. s. **41**: 872–874. 1915. Livingston, B. E., and Hawkins, Lon A., The water-relation between plant and soil. Carnegie Inst. Wash. Pub. **204**: 3–48. Washington, 1915.

[6] Livingston, B. E., and Hawkins, Lon A. (1915). Pulling, H. E. and Livingston, B. E., The water supplying power of the soil as measured by osmometers. Carnegie Inst. Wash. Pub. **204**: 49–84. Washington, 1915.

Temperature conditions. Various attempts have been made from time to time to derive an index to represent the temperature conditions as these may affect plant growth, and three general methods have so far been suggested to accomplish this. These three methods have been discussed in a previous paper,[10] with some reference to earlier literature. The earliest one to be employed is the differential method, according to which plants are supposed to grow at a rate proportional to the number obtained by subtracting a constant (as 39°, 40°, 42°F., etc.) from the mean temperature for the period considered. Thus if the mean temperature of a day were 75°F., growth might be considered to go forward on that day at a rate of 36 $(75-39)$ when the growth-rate for a day having a mean temperature of 40°F. is considered as unity $(40-39)$. The exponential method was devised on the supposition that plant growth may proceed at rates proportional to the velocities of those chemical reactions that about double in rate for each rise of 10°C. (18°F.). The physiological method was derived by employing the results of Lehenbauer's[11] actual measurements of the rate of elongation of young maize shoots as influenced by temperature. This method, described in the paper on temperature indices just mentioned, is the only one so far brought forward that attempts to take account of the optimum and maximum of temperature as related to plant growth.

By all three methods summation or integration is resorted to when considerable time periods are involved, and a summation index of temperature efficiency is finally obtained, which aims to represent the temperature conditions as these may have affected plant growth throughout the period. Thus, if a month, the growing season, or the year, etc., be considered, the mean temperature for each day (hour, etc.) within the period is first determined. Then the efficiency index corresponding to each of these means is found, according to either one of the three methods, and these indices are then summed to give the summation index for the period.

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[7] Transeau, E. N., Forests of eastern America. Amer. Nat. **39**: 875–898. 1905. *Idem*, Climatic centers and centers of plant distribution. Mich. Acad. Sci. Ann. Report **7**: 73–75. 1905.

[8] The arithmetical *difference* between depth of evaporation from a specified kind of atmometer pan and depth of rainfall, for any period is not to be regarded as a logical measure of moisture conditions, though many climatologists appear still to consider this "deficit" as of some value. A consideration of the question thus raised is postponed, for a later paper.

[9] Ecologists, agriculturalists and foresters should not be unmindful of the possibility of employing the ratio of soil moisture *content* to evaporating power of the air, for a suitable length of season, wherever the limited extent of the geographic area studied and the available facilities allow frequent determination of the soil moisture content. This ratio was first suggested by Shreve, who has employed it with very satisfactory results in vegetational-climatic studies of the Santa Catalina Mountains, in Arizona. See: Shreve, F., Rainfall as a determinant of soil moisture. Plant World **17**: 9–26. 1914. *Idem*, The vegetation of a desert mountain range as conditioned by climatic factors. Carnegie Inst. Wash. Pub. 217. Washington, 1915. Pages 92, 93.

[10] Livingston, B. E., Physiological temperature indices for the study of plant growth in relation to climate. Physiol. Res. **1**: 399–420. 1916.

[11] Lehenbauer, P. A., Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914.

Light, chemical and mechanical conditions. Though light conditions have attracted the attention of many students of plant growth, no wholly satisfactory method for their measurement, as they may affect plants, has yet been brought forward. Progress seems to be being made in this direction however. Chemical and mechanical conditions have not yet been studied in nature from this point of view.

The environmental complex. While environmental conditions may be classified in groups, as above outlined, it is to be emphasized that such classification is quite artificial and is resorted to only in the interest of convenience. The different kinds of conditions do not influence plants one at a time, but the whole environment acts singly upon the plant, the various separate conditions being the component parts of the environmental complex. One of the final aims of physiological ecology may therefore be to gain sufficient appreciation of environmental relations so as to be able to describe the whole environment by a single quantitative measure, which might express the complex in terms of its effectiveness to promote plant growth. As in other scientific enquiries regarding the conditioning of complex natural phenomena, the logical procedure must be, first to analyze the complex into its component elements, then to study each element separately, and finally to find mathematical expressions by which the various elementary measurements may be combined to represent the complex as a whole.

This procedure is being followed, more or less consciously, by many workers in plant physiology and its applications, but, as has been pointed out, the last three of the five groups of conditions are not yet susceptible of measurement in terms such as to be applicable to the interpretation of plant growth. With the moisture and temperature conditions however, measurements of a certain degree of value can now be made, and it is with an attempt to combine such measurements into a partial index of environmental efficiency for plant growth that this paper deals.

That this attempt is not entirely premature and ill-advised may be argued on several grounds. First, moisture and temperature conditions are more frequently met with as conditions limiting the geographical distribution of wild and cultivated plants than are any of the other groups. So far as we now know, it is only in comparatively restricted areas that light, chemical or mechanical conditions prevent the occurrence of any given plant form in nature. The terms representing moisture and temperature may therefore be expected to occupy a very prominent place in the environmental formula just suggested, and the tentative construction of such a formula may well begin with these terms. Second, an early attempt to derive a moisture-temperature index of environmental efficiency may possibly throw some light on actual problems of ecology, agriculture and forestry; at any rate, such an attempt should be of value in showing what sorts of studies of the elementary conditions are most apt to be useful and what sorts are least promising. Third, the attempt here made may possibly accelerate progress in this fundamentally important field merely by emphasizing the goal toward which it is directed, being thus a plea for a more wide-spread interest in the deeper and broader problems of ecology.[12] Other lines of argument might be advanced.

A SINGLE INDEX OF MOISTURE-TEMPERATURE EFFICIENCY FOR PLANT GROWTH

The index here suggested is simply the product obtained by multiplying Transeau's rainfall-evaporation ratio for the period in question by the summation index of temperature efficiency for the same period. Since the physiological indices of temperature efficiency, derived from Lehenbauer's results, appear to be most thoroughly grounded both in theory and in fact, they are to be recommended in preference to the other temperature efficiency indices.

The rainfall-evaporation ratio may be regarded as a rough first approximation to an index of the moisture conditions in general, as has been indicated. It is not nearly as well grounded as are the physiological temperature indices, and it will surely be modified in several ways. As to its derivation, it is of course obtained for any period by dividing the rainfall index by the index of the evaporating power of the air, both for the period in question. But it must be borne in mind, in this connection, that evaporation is a practically continuous process, while precipitation for many hours and days in most regions is usually nil, its rate being high for short periods of time and zero for the much longer intervening periods.

To obtain an index of rainfall for a comparatively short period of time (as for an hour, a day, etc.) it is necessary to devise some conventional method by which the spasmodically jerky graph of rainfall may be smoothed. The simplest way of accomplishing this is to distribute the rainfall value of each shower evenly over a period of time reaching from the beginning of that shower to the beginning of the next shower. Practically, the day is usually the time increment considered, rather than the hour, and it will here be employed when examples are given. If a rainfall of 5 cm. occurred on July 1 and it rained no more till July 7, the value 5 would be divided by 6, and the rainfall index for each day from July 1 to 6, inclusive, would be taken as 0.83. It is obviously incorrect, in the above example, to give to July 6 as high a daily value as is given to July 1, but methods for the weighting of rainfall values according to their possible effectiveness in promoting plant growth have never yet been reported in the literature, and its consideration will not be taken up here. After obtaining a value for each day of the entire period considered, these values are summed to give an index for the period. For long periods (as months, seasons or years) the total amount of rainfall occurring within the period is simply summed to give the rainfall index.

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[12] It has long been my conviction that if the term ecology is to be retained its meaning should include the scientific aspects of agriculture, forestry, etc. Many writers seem to restrict the meaning to the study of wild plants, but this restriction is too artificial to be anything but a stumbling-block to clear thinking. A man with a hoe, killing some plants and letting others develop, is as much a natural phenomenon as is a local shower, the falling of an old tree in the forest, or the erosion of a stream bank, all of which may also kill some plants and lead to the better development of others. It has always seemed odd to me that scientific students of plants should so frequently exhibit great interest, for example, in the distribution of seeds by birds or of pollen by insects, and at the same time profess no interest in the distribution of seeds, etc., by man and his machines.

To obtain the index of the evaporating power of the air for any period, the total amount of water lost from the given standard atmometer during the period is employed. Here, again, no method for weighting evaporation values has yet been suggested. It is assumed that plant growth is affected by rainfall and by atmospheric evaporating power proportionally to the summations obtained.

It is not to be ignored, however, that rainfall generally acts to promote plant growth and evaporation acts to retard it (as far as the water relation alone is concerned). If, then, we consider the rainfall values as directly proportional to the influence of this condition in promoting plant growth, we shall be obliged to consider the *reciprocals* of the evaporation values as similarly proportional. (See Livingston and Hawkins, 1915, already cited.) If the rainfall value for July 1, in the example given above, be 0.83 and the evaporation value for the same day be 20, the efficiency of these two conditions in producing or accelerating plant growth is taken as $0.83 \times \frac{1}{20}$, or $\frac{0.83}{20} = 0.0415$. On the following day, still following the above illustration, we should assume the same value for precipitation and divide it by the observed value for evaporation, etc. For long periods total precipitation may be divided by total loss from the atmometer, to obtain the required tentative index of efficiency of the moisture conditions as a whole.

The units used in measuring rainfall and evaporation are not here important, so long as these do not vary throughout any investigation, but it is to be emphasized that the kind of atmometer used must be the same at all stations compared, for the value of any unit changes with the instrument. Thus a cubic centimeter of evaporation from a cylindrical porous cup is not at all equivalent to a cubic centimeter lost from a pan of water, although these two units may appear alike.[13]

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[13] This question of atmometric units seems to require special treatment, with experimental data, for the matter appears not to be self-evident to every one. Such a discussion may be postponed, however.

Having obtained the rainfall-evaporation ratio for a given period, the next step in obtaining the moisture-temperature index is to multiply this ratio by the corresponding summation index of temperature efficiency. The method of obtaining this last index has been described above.

It remains to scrutinize the moisture-temperature index, to understand upon what sort of assumptions it rests and how it is to be interpreted. As has been indicated, the three primary assumptions are (1) that plant growth is accelerated proportionally to the value of the rainfall index, (2) that it is retarded proportionally to the index of the evaporating power of the air, and (3) that it is accelerated proportionally to the index of temperature efficiency. In the third case alone has any system of weighting been applied and the weightings there employed are based on experiments with young maize seedlings exposed for 12 hours to maintained temperature. The fundamental nature of these assumptions shows how exceedingly tentative the derived product must be. When weightings for rainfall and evaporation readings, and for the moisture ratio, are obtainable, and when the temperature weightings (which I have derived from Lehenbauer's work alone) have been subjected to the correction that they will surely undergo, then the conception of the final product may be expected to become less tentative.

The index of moisture-temperature efficiency as above described may be represented by the formula

$$I_{mt} = I_t \frac{I_p}{I_e},$$

where I denotes the efficiency index for the time period considered and the subscript letters denote the respective environmental conditions for which the various indices stand. I_{mt} is the moisture-temperature index[14] with which we are mainly concerned. I_t is the index of temperature efficiency, derived by means of the physiological system. I_p is the index of precipitation intensity and represents simply the summation of the rainfall for the period. I_e is the index of the atmospheric evaporating power, also a simple summation for the period.

Inspection of the formula just stated shows that the value of the *moisture-temperature* index is increased by higher temperature (so long as the optimum for plant growth is not passed) and by the length of the period considered. The higher the daily index values and the more of them summed, the greater must be the resulting sum. Also, the product index is increased by higher values of the moisture ratio. This ratio, in turn, is increased by more rainfall and by lower atmospheric evaporating power. The efficiency of the moisture-temperature complex is thus greatest with a long season, with high temperatures (not above the optimum), with great rainfall and with low evaporating power.

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[14] It has been suggested by Mr. E. E. Free that the Greek word *hydrothermal* may be substituted or employed as a synonym for *moisture-temperature*. The Greek term should be clear to every one and it avoids a certain homely awkwardness evident in the other. If others should become interested in this product their usage may be allowed to determine what it will at length be called.

When the daily mean temperatures (they may of course be hourly, etc.) surpass 89.6°F. the physiological system of temperature indices here employed renders the index values lower than that for 89.6° (see Livingston, 1916, already cited). Thus, a daily mean temperature of 100°F. is taken as having the same efficiency as one of about 72.5°F., both of these efficiency values being about 67 times as great as that for 40°F. The temperature 89.6°F. is considered as the optimum for plant growth.

AN EXAMPLE OF THE USE OF MOISTURE-TEMPERATURE INDICES IN CLIMATOLOGY

One of the numerous ways in which the new moisture-temperature index may be employed is in connection with the study of climatic zones with reference to the distribution of vegetation, agricultural crops, etc. Here the environmental conditions of any single year or season are not the center of interest, but the climatic *normals* (or averages for a number of years) are the subject of study. In the present instance an attempt is made to obtain a chart of the United States to show the normal distribution or zonation of moisture-temperature efficiency throughout the country, for the period of the average frostless season. The average frostless season is here taken to represent the average season of active growth of plants.

The data on which the computations are based are derived from publications of the U. S. Weather Bureau. The average frostless seasons for the stations considered are those given in my earlier paper on physiological temperature indices [1916], and were derived from average frost data published in the Summary by Sections.[15] For precipitation, the normal daily rainfall for each station was obtained from Bigelow's[16] tables of these data. The only available evaporation values at all suited to our purpose, for the United States, are the monthly data obtained by Russell[17] for the year July, 1887, to June, 1888. These are not normals, but it seems best to employ what data we have in this connection rather than to wait for better, especially since it must take years of observation before normals of the evaporating power of the air may be hoped for, and since this kind of observation has not yet been begun.[18]

[15] Summary of the climatological data of the United States, by sections. U. S. Dept. Agric., Weather Bureau. Washington, no date.

[16] Bigelow, F. H., The daily normal temperature and the daily normal precipitation of the United States. U. S. Dept. Agric., Weather Bur. Bull. R. Washington, 1908.

[17] Russell, T., Depth of evaporation in the United States. Monthly Weather Rev. **16**: 235–239. 1888.

[18] Had evaporation observations at the regular stations of the U. S. Weather Bureau been begun after Russell's experiment was completed and had they been continued regularly to the present time, we should now be in possession of a very satisfactory series of atmometric normals.

Russell's data are given by months instead of by days, so that special computations were required to obtain the evaporation value for the period of the average frostless season. This period, for each station, consists of a certain number of whole months, and of two fractions of months, one of the latter at the beginning and the other at the end of the period. The value given by Russell for each of these two months that are only partly included in the period was multiplied by the number of days of that month so included and the product was then divided by the total number of days in the month. The two values thus obtained, together with those for the whole months within the period, were then summed, giving approximations of the total evaporation for the period. Russell's data were not observed but calculated. It is unfortunate that the series represents parts of two summers, though this odd feature does not make them much worse for the present purpose than would be the case had a single calendar year been dealt with.

The summation indices of temperature efficiency are taken from my paper [1916] on this subject, the normal daily mean temperatures having been obtained from Bigelow's tables already cited. The temperature data themselves are Fahrenheit, since the U. S. Weather Bureau still employs these.

Table I presents a list of the stations considered, the values of the moisture ratio, $\frac{I_p}{I_e}$, and those of the moisture-temperature index, I_{mt}. For the lengths of the average frostless seasons and for the summation indices of temperature efficiency, I_t, reference may be made to the paper on the latter already cited.

The values of the moisture-temperature index for the period of the average frostless season, given in table I, show a very broad range; the smallest value is 197 (Winnemucca, Nevada) and the largest is 23,266 (Key West, Florida). The range thus shown is from unity to 118. In order to study the geographical relations of these various values and to bring out the climatic zones indicated by these relations, all the values were plotted upon a map of the country and isoclimatic lines[19] were drawn in the usual manner. The resulting chart is here reproduced as figure 1. The stations employed are indicated by dots. The numbers on the isoclimatic lines represent thousands.

The chart of figure 1 exhibits several features not shown by the charts of most climatic indices. As is to be expected from the nature of the indices here employed, high values occur where the supply of both heat and moisture is great during the period of the average frostless season. If this period is

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[19] If the moisture-temperature indices are termed *hydrothermal*, these lines become *isohydrothermal* lines, or *isohydrotherms*, and the chart itself is a *hydrothermal chart*, or a chart of *hydrothermal conditions*, following various analogs in climatology. The convenience of these expressions is in favor of the adoption of the Greek expression for moisture-temperature, but I have not thought it best to employ the latter in the present early stage of this sort of study. It seems best to allow these new ideas and concepts some time in which to develop, before special technical terms are thrust forward.

TABLE I

Rainfall-evaporation ratios and moisture-temperature indices for the period of the average frostless season, for 170 stations in the United States

STATION	LENGTH OF AVERAGE FROSTLESS SEASON	RAINFALL EVAPORATION RATIO FOR PERIOD OF AVERAGE FROSTLESS SEASON	MOISTURE-TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROSTLESS SEASON
	days		
Alabama			
Mobile	279	1.36	23652
Montgomery	243	0.75	12400
Arkansas			
Fort Smith	230	0.76	10782
Little Rock	237	0.77	11246
California			
Fresno	258	0.08	1186
Independence	222	0.04	449
Los Angeles	334	0.37	3127
Red Bluff	264	0.17	2409
Sacramento	272	0.21	2046
San Francisco	319	0.48	1991
Colorado			
Denver	153	0.19	1204
Connecticut			
New Haven	180	1.17	7869
Florida			
Jacksonville	293	1.16	21760
Key West	365	0.75	23266
Pensacola	285	1.08	20465
Tampa (Cedar Keys*)	335	1.08	23155
Georgia			
Atlanta	225	0.74	9686
Augusta	228	0.85	12879
Savannah	263	1.12	18294
Idaho			
Boise	177	0.09	598
Illinois			
Cairo	212	0.64	7807
Chicago	182	0.74	5100
Springfield	182	0.74	7032
Indiana			
Indianapolis	186	0.63	5967
Iowa			
Davenport	174	0.74	6255
Des Moines	171	0.89	7457
Dubuque	176	0.95	7472
Keokuk	197	0.75	7241

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* Where a second station is named in parenthesis, the evaporation value is for this station.

TABLE I—Continued

STATION	LENGTH OF AVERAGE FROST-LESS SEASON	RAINFALL EVAPORATION RATIO FOR PERIOD OF AVERAGE FROST-LESS SEASON	MOISTURE-TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROST-LESS SEASON
	days		
Kansas			
Concordia	173	0.70	7114
Dodge	181	0.43	4474
Topeka	189	1.01	10599
Kentucky			
Louisville	196	0.57	6590
Louisiana			
New Orleans	310	1.21	23381
Shreveport	252	0.82	13874
Maine			
Eastport	167	1.31	2747
Portland	157	1.04	4528
Maryland			
Baltimore	213	0.76	7947
Washington, D. C	197	0.83	8322
Massachusetts			
Boston	185	0.93	5714
Nantucket	209	1.03	5193
Michigan			
Alpena	137	1.01	3300
Detroit	164	0.69	4569
Grand Haven	167	0.79	4189
Marquette	140	1.03	3113
Port Huron	155	0.78	3819
Minnesota			
Duluth	152	1.23	4064
Moorhead	132	0.94	4043
St. Paul	159	1.03	6423
Mississippi			
Vicksburg	252	0.93	15125
Missouri			
Kansas City (Leavenworth, Kans.*)	196	0.86	8875
St. Louis	207	0.58	6824
Springfield	187	1.00	10061
Montana			
Havre (Fort Assiniboine*)	122	0.37	1505
Helena	144	0.22	809
Nebraska			
North Platte	151	0.51	3682
Omaha	170	0.82	7406
Valentine	132	0.60	3861

TABLE I—Continued

STATION	LENGTH OF AVERAGE FROST-LESS SEASON	RAINFALL EVAPORATION RATIO FOR PERIOD OF AVERAGE FROST-LESS SEASON	MOISTURE-TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROST-LESS SEASON
	days		
Nevada			
Winnemucca	131	0.04	197
New Hampshire			
Concord (Manchester*)	146	0.94	4422
New Jersey			
Atlantic City	207	1.30	10241
New Mexico			
Santa Fé	187	0.18	979
New York			
Albany	177	0.84	5598
Buffalo	173	0.78	4511
New York	210	0.87	7034
Oswego	175	0.87	4784
Rochester	171	0.71	4100
North Carolina			
Charlotte	220	0.88	11022
Hatteras	256	1.76	24265
Raleigh	213	1.22	14980
Wilmington	233	1.35	18240
North Dakota			
Bismarck	129	0.55	2626
Devils Lake (Fort Totten*)	121	0.75	2823
Williston (Fort Buford*)	119	0.42	1902
Ohio			
Cincinnati	194	0.51	5513
Cleveland	198	0.78	5606
Columbus	184	0.58	5112
Sandusky	195	0.74	5778
Toledo	174	0.62	4647
Oregon			
Portland	245	0.66	3160
Roseburg	198	0.29	1313
Pennsylvania			
Erie	194	0.90	6047
Philadelphia	206	0.75	7010
Pittsburgh	179	0.66	5672
Rhode Island			
Block Island	218	1.39	7555
South Carolina			
Charleston	276	1.16	19608
Columbia	231	0.98	14837

TABLE I—Concluded

STATION	LENGTH OF AVERAGE FROST-LESS SEASON	RAINFALL EVAPORATION RATIO FOR PERIOD OF AVERAGE FROST-LESS SEASON	MOISTURE-TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROST-LESS SEASON
	days		
South Dakota			
Huron	131	0.66	3687
Pierre (Fort Sully*)	153	0.39	2928
Yankton	154	0.91	6961
Tennessee			
Chattanooga	207	0.81	10052
Knoxville	208	0.82	8970
Memphis	224	0.75	10837
Nashville	207	0.69	8930
Texas			
Abilene	245	0.44	7028
Amarillo (Fort Eliot*)	199	0.44	4673
Corpus Christi	298	0.65	13926
El Paso	236	0.12	1790
Galveston	331	0.97	20570
Palestine	245	0.79	12977
San Antonio	276	0.51	9716
Utah			
Salt Lake City	182	0.13	1052
Vermont			
Northfield	126	1.16	3884
Virginia			
Lynchburg	201	0.87	9228
Norfolk	230	1.24	15060
Washington			
North Head (Fort Canby*)	316	0.70	1874
Spokane	202	0.23	1148
Tatoosh Island	271	3.84	7475
Walla Walla	216	0.18	1475
Wisconsin			
Green Bay	153	0.86	4270
La Crosse	163	0.94	6296
Milwaukee	162	0.87	4582
Wyoming			
Cheyenne	119	0.20	710

somewhat deficient in heat supply or in moisture supply this deficiency is overcome if the other factor of the index is very large. Where both factors are small the index value must be correspondingly small. This brings it about that the chart partakes of the character of a temperature chart and also of that of a chart of moisture conditions. In the west it has the appearance of a moisture chart, in the east it resembles a chart of tempera-

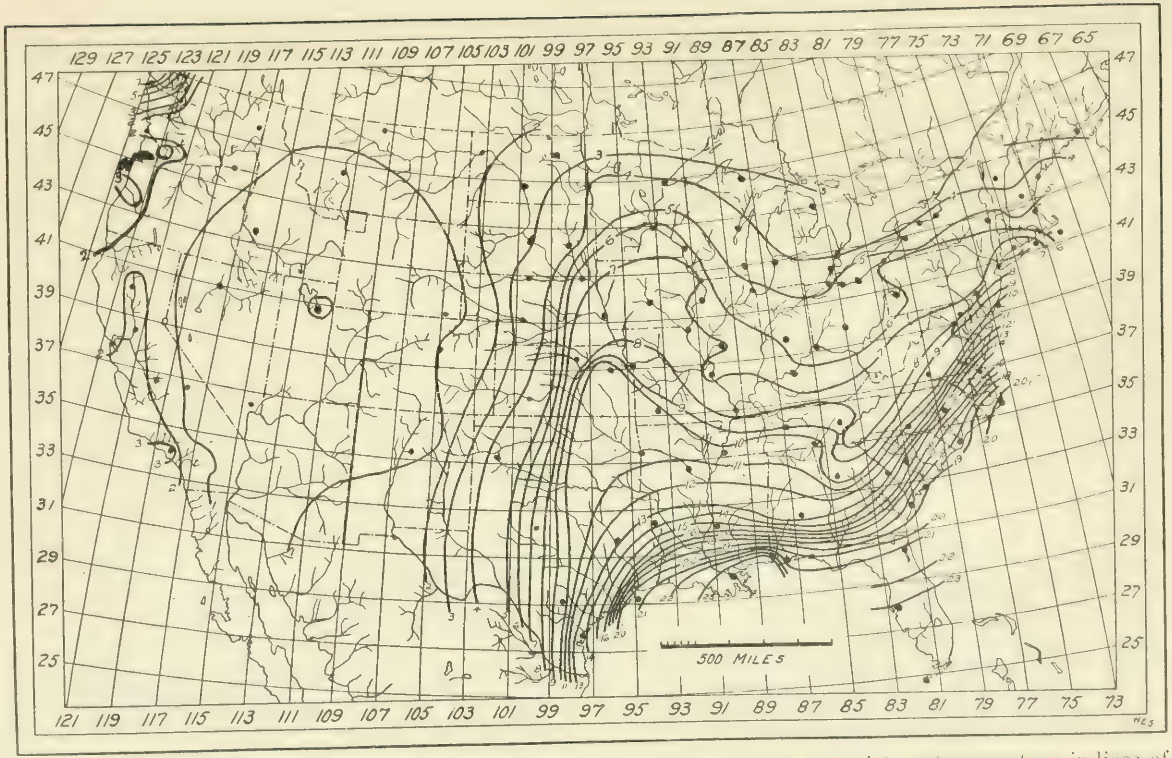

Fig. 1. Chart of the United States showing climatic zonation according to moisture-temperature indices of climatic efficiency for plant growth, for the period of the average frostless season. The numbers on the isoclimatic lines each represent thousands.

ture, and the well-known climatic differentiation between the regions lying west and east of about the hundredth meridian is clearly indicated. The highest values occur in the southeast and the lowest ones are found in the desert region. The overwhelming influence of very low moisture indices is brought out by the fact that the region of low moisture-temperature values extends southward into Mexico in spite of the high temperature indices that characterize its southern portion, while the region of high moisture-temperature values does not extend northward in a corresponding way. From this it might be stated that aridity is the controlling climatic condition in the arid region while temperature is the controlling one in the humid region. Most of these generalizations are indicated by other climatic charts, but heretofore it required two charts, and some reasoning, to make them evident.

To students of physiological ecology, whose vegetational zones have been founded on the general moisture requirements of plants as these are reflected in the appearance or structure of the latter, the climatic zonation of the present chart will seem bizarre, anomalous and even impossible of logical interpretation. If it be supposed that these moisture-temperature indices do indeed measure a climatic dimension that is related to plant growth, how can it be that Portland, Maine; Milwaukee, Wisconsin; Dodge, Kansas; and Amarillo, Texas, are all represented as lying in the same climatic zone? Obviously the appearance of the natural vegetation is not at all the same throughout this zone, and it may thus appear that moisture-temperature indices must be quite without value in the study of climate as related to vegetational distribution. But this apparent discrepancy vanishes with further analysis of the proposition.

The obvious physiological characters of plants are related to the moisture conditions alone; one cannot tell by inspecting a plant what length of growing season or what temperatures it may require, but one can frequently make a fairly reliable estimate of its moisture requirements on the basis of appearance. Therefore, the obvious climatic feature visibly depicted by vegetation is the moisture index alone.

Furthermore, the amount of plant growth possible in a year at any locality is not only dependent upon moisture and temperature intensity during the growing season, but it is also influenced by the duration factor of the environmental complex, the length of the season of active growth,[20] while the visible character of the vegetation is not related to the length of the season of growth at any place in the United States—unless it be at very high altitudes. (In northern Canada a broad desert region includes the whole width of the continent, without any influence of the moisture conditions.) On the other hand, the moisture-temperature indices do include a duration factor representing the length of the period of active growth.

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[20] Of course the natural vegetation of a given locality, as well as the cultivated plants, are influenced, in both their physiological and floristic characters, by conditions that are effective at other periods than that of the growing season, and winter conditions clearly need to be considered in attacking the problem of plant distribution. These are intentionally neglected here, for the sake of simplicity.

On the whole, then, for these and other reasons, it is not to be expected that such a climatic measure as the one here under consideration should exhibit features very clearly in accord with the apparent features of natural vegetation.

Cultivated plants furnish a clue, however, to the interpretation of the chart before us. The short season of growth and the comparatively low temperature of this season at Portland, Maine, render the climatic efficiency of this region low for the year as a whole, but the moisture conditions at Portland are known to be quite adequate for excellent plant growth the year round. On the other hand, the temperature conditions for the year at Amarillo are apparently little better than those at Washington, D. C., but the low rainfall and high atmospheric evaporating power give to the moisture-temperature index for the western station a value only about half as great as that for Washington. The duration factors are about the same for these two stations (199, 197 days), and one way to obtain as much annual plant product from an acre of land at Amarillo as is naturally possible at Washington would be to improve the moisture conditions at the western station till they were about the same as those for the eastern. This can be accomplished artificially, by irrigation, which virtually increases the rainfall and also tends to decrease the evaporating power of the pair over the irrigated land. Another way to improve the moisture-temperature conditions at Amarillo and render them equal to those at Washington would be to lengthen the period of the average frostless season at the former place. This sort of modification of climate has actually begun to be accomplished in some regions, by covering the plants—with cloth covers in some cases, with a smoke blanket in others—on a few frosty nights at the end of the frostless season.

If it were possible to improve the temperature conditions (length and temperature-efficiency of the season of plant growth) for Portland until they were as good as those for Tampa, Florida, then the potential annual plant product per acre for the northern station should about equal that for the southern. But temperature conditions are not as easily controlled by artificial means as are moisture conditions; it is much easier to make a desert moist than to make winter into summer. So it comes about that large areas of the arid southwest are annually producing as much as their temperature conditions allow, while only exceedingly small areas in the Portland region are producing as much per year as their moisture conditions might allow. These latter areas are of course under glass; greenhouses are the only localities where the natural winter is transformed into an artificial summer. This argument can be made more precise when a light index becomes available, light being neglected, perforce, in all of the present discussions.

In this sort of comparison of climates it is advantageous to carry the analysis one step further and to derive the mean daily index of temperature efficiency for the period of the average frostless season, by dividing the summation temperature index by the number of days in the season. This allows the temperature factor of the moisture-temperature index to be considered as the product of two factors, one of duration and the other of intensity. The former is the length of the average frostless season, the latter is the average daily index of temperature efficiency for the season. Some of the features brought out by a consideration of all the factors so far available are shown by the sample data given in table II.

This table shows that Amarillo and Washington are about alike in length of average frostless season (199, 197) and also in daily mean temperature efficiency (54, 51). They are unlike as to both rainfall and evaporation but the difference between their moisture ratios is due more to difference in rainfall than to difference in evaporation. Portland and Los Angeles have daily mean temperature indices about alike (28, 25). The former has a short average frostless season (157) and the latter a long one (334), but this difference in favor of the moisture-temperature efficiency for the western station is much more than counterbalanced by the fact that the moisture ratio here (0.37) is only about one-third as large as that for the eastern station (1.04). The evaporation index for Los Angeles (35) is not much larger than that for Washington (30), but the precipitation index for the western station is very low. This throws light upon the fact that irrigation is so effective in the region of Los Angeles. On the other hand, the mean daily temperature index for Washington (51) is twice as large as that for Los Angeles (25) but

TABLE II

Numerical climatic data for six selected stations in the United States

STATION	DURATION OF AVERAGE FROSTLESS SEASON (D)	NORMAL RAINFALL FOR PERIOD OF AVERAGE FROSTLESS SEASON (I_p)	INDEX OF ATMOSPHERIC EVAPORATING POWER FOR PERIOD OF AVERAGE FROSTLESS SEASON (I_e)	MOISTURE RATIO FOR PERIOD OF AVERAGE FROSTLESS SEASON $\left(\frac{I_p}{I_e}\right)$	PHYSIOLOGICAL TEMPERATURE EFFICIENCY INDEX FOR PERIOD OF AVERAGE FROSTLESS SEASON (I_t)	DAILY MEAN TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROSTLESS SEASON $\left(\frac{I_t}{D}\right)$	MOISTURE-TEMPERATURE INDEX FOR PERIOD OF AVERAGE FROSTLESS SEASON (I_{mt})
	days	*inches*					
Amarillo, Tex.	199	17.5	40.0	0.44	10668	54	4673
Washington, D.C.	197	25.0	30.3	0.83	10087	51	8322
Portland, Me.	157	17.8	17.1	1.04	4362	28	4528
Tampa, Fla.	335	50.1	46.4	1.08	21420	64	23155
Milwaukee, Wis.	162	16.6	19.1	0.87	5261	32	4582
Los Angeles, Calif.	334	12.9	34.8	0.37	8451	25	3127

the Los Angeles frostless season (334) is 1.7 times as long as the Washington season (197). The very high moisture-temperature index shown for Tampa is seen to be due to a long season and to a high mean daily temperature index, as well as to a high moisture ratio. It is interesting to note that the index of atmospheric evaporating power for Tampa (46) is greater than for Amarillo (40) and much greater than for Los Angeles (35). But this is more than counterbalanced by the high precipitation index for Tampa (50.1). Tampa and Los Angeles are alike as to length of the average frostless season (335, 334). Many other significant features of these six climatic complexes might be mentioned.

According to the chart of figure 1 it appears that the most efficient climate for plant growth, in the United States, is that of peninsular Florida, as far as moisture and temperature conditions are concerned. But this climate is not the most comfortable for human beings; its moisture ratio is too high in the season of active plant growth. The reason why the climate of southern California is so generally regarded as better than that of Florida is to be found in the facts, (1) that the moisture ratio here is very low (making it a very poor climate for plant growth but a very pleasant one for human beings, and (2) that the moisture ratio is here artificially raised for plants (by irrigation), but not thus generally raised for human beings. The southern California climate for cultivated plants is an artificial one, in as true a sense as is that of a greenhouse in winter in Maine. In the latter case the value of the temperature index is artificially increased.

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DEPRESSION OF THE FREEZING-POINT IN TRITURATED PLANT TISSUES AND THE MAGNITUDE OF THIS DEPRESSION AS RELATED TO SOIL MOISTURE

R. P. HIBBARD AND O. E. HARRINGTON

ABSTRACT[1]

This paper deals with the freezing-point lowering that characterizes the pulpy mass formed by grinding plant tissues, and presents evidence that this lowering is as valuable a criterion for comparing osmotic concentrations of the tissues as is the corresponding index for the expressed juice. The material is first subjected to a preliminary freezing, to render the cell membranes more readily permeable to dissolved materials, after which it is thoroughly triturated. The pulp thus prepared is placed in the cryoscopic apparatus and its freezing-point depression is determined just as is usually done with the sap expressed from such pulps. Beckmann's freezing-point apparatus was employed.

It was found that this method of testing the pulp without pressing gives concordant results when different samples of the same pulp are tested. In a series of duplicate tests the greatest plus or minus variation between two lowerings that one might expect to be alike was only 0.5 per cent; usually it was less than this.

Comparisons of the depressions obtained from tests of plant pulps, with those from tests of the expressed juices of the same pulps, show that the two values obtained in these two ways were practically identical, providing that the process of pressing had been very thorough. The agreement is usually within much less than 1.0 per cent. When pressing is far from complete these two values do not agree, but this is regarded as due to incomplete pressing. It is therefore concluded that the pressing out of the juice for such determinations is unnecessary and may be omitted. Thus the method for determining the freezing-point depression and osmotic concentration of ground or crushed tissues is markedly simplified. Of course it is applicable only when a considerable quantity of pulp is available.

The materials employed in these tests were: potato tubers, cabbage leaves (from the head), apples, lemons, oranges, grape-fruits, onion bulbs, and the tops and roots of maize plants grown in pot cultures.

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[1] The manuscript of this paper was received June 15, 1916. This abstract was preprinted, without change from these types and was issued as Physiological Researches Preliminary Abstracts, vol. 1, no. 10, August, 1916.

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In the case of the maize plants the pots were furnished with Livingston auto-irrigators, arranged so that six different degrees of soil moisture were practically maintained, in as many different cultures. When the plants were 7 weeks old the roots were removed from the soil, separated from the tops, externally dried, frozen, ground and finally subjected to the test for freezing-point lowering. The tops (leaves and stems together) were also tested. It was found that the triturated tops showed depressions of from 1.835°C. (culture with mean soil moisture of 31 per cent on dry weight) to 2.204°C. (culture with soil moisture of 11 per cent), and that the ground roots showed corresponding depressions of from 0.492° to 0.995°C. The root material is thus seen to have had a much lower depression (and consequently a much lower osmotic concentration) than the top material. It is especially important to note that the depression of the freezing-point was found to increase, for both tops and roots, as the moisture content of the soil in which the plants grew decreased, from culture to culture in the series. The order of magnitudes was the same for both roots and tops, being the reverse of the order for soil moisture content.

INTRODUCTION

There are three indirect methods by which may be determined the osmotic concentration of the liquid obtained by grinding plant tissues. These employ, respectively, vapor pressure, lowering of the freezing-point, and raising of the boiling-point. The conductivity method, though simple and convenient, can not be used, since non-electrolytes, colloids and suspended particles are present in such plant saps and make it impossible to regard electrical conductivity as a measure of osmotic concentration. In fact there is no relation between the lowering of the freezing-point and electrical conductivity values, according to Nicolosi, as cited by Bottazzi.[2] Of the three possible methods, that employing the freezing-point finds greater application than the others, on account of its comparative simplicity and ease of manipulation, and it is with this method that the present paper deals.

It must be observed that any method employing ground tissues does not give the actual osmotic pressure effective in the cell, for the properties of the membranes and the water supply take part in determining this actual pressure. The freezing-point method appears to give an approximation, however, of the maximum osmotic pressure possible in any tissue, if plenty of water is supplied and if the cell membranes are quite permeable to it and impermeable to the solutes. This statement is based on the supposition that the osmotic concentration of the sap when frozen is quite similar to that of the vacuolar sap as it existed in the uninjured cells.

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The determination of the osmotic concentration of the sap of ground plant tissues by the freezing-point method dates back to 1886, at least, when Müller-Thurgau[3] determined the freezing-point lowerings of certain ground plant parts, such as potato tubers, apple and pear fruits, grapes and leaves of the common bean. In some cases the juice was expressed, in others he triturated the plant tissues and then found the freezing point of the resulting mash. Sometimes he cut openings into the otherwise intact tissue and inserted the thermometer, afterwards freezing the entire piece.

Maquenne[4] obtained cryoscopic measurements upon the expressed juices of sunflower and pea seedlings, and Sutherst[5] determined the freezing-points of the juices expressed from tissues of vegetable marrow, turnip, celery, carrot, cabbage, apple and pear.

In 1905 Cavara[6] made extended studies on the freezing-points of expressed plant saps of many species and varieties, and of many plant parts at different stages of development, thus obtaining results of great value to the ecologist. The tissues were pressed in a porcelain press and strained through a fine linen cloth. When it was difficult to extract the juice, because of its thick, gummy consistency, he obtained the freezing-point lowering of the mash, or, in some cases, froze the whole organ, as Müller-Thurgau had done. One of Cavara's conclusions that is of particular interest, and one that applies in the present study, is that the osmotic concentration as thus measured varies from plant to plant, even in the same species when the different individuals are of different ages, have been grown under different conditions, etc.

In the following year Livingston[7] found, by use of both the freezing-point and boiling-point methods, that the expressed juices of certain cacti exhibited osmotic pressures no higher than those commonly found in plants of the humid region. He also encountered the same difficulty as the earlier workers, when tissues of a gummy, mucilaginous consistency were being prepared for the cryoscopic test.

Drabble and Drabble[8] drew attention to the relation of cell sap concentration to the physical environment of the plant, expressed juices and the cryoscopic method being used.

During the past eight or nine years this line of work has been considerably extended. Though Fitting[9] and Gola[10] did not use the freezing-point method they have added considerable to our knowledge of the ecological relationships between the apparent osmotic pressures of plants and the soil solutions in which they live.

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[10] Gola, F., Saggio di una teoria osmotica dell' edafismo. Annali Bot. **3**: 455-512. 1905.

In 1910 Atkins[11] published some data obtained by cryoscopic determinations of plant saps. In 1912 Marie and Gatin[12] made use of the cryoscopic method in their work on the sap concentration of alpine plants.

Dixon and Atkins[13] greatly improved the method of preparing material for the freezing-point determination. Instead of crushing or grinding and then pressing the material, it was first frozen in liquid air, then quickly transferred to a stoppered receptacle and allowed to stand until the tissues had come to the surrounding temperature, after which it was ground and pressed in the usual way. The authors also devised a new, thermo-electric method for determining the freezing-point. The chief value of this method lies in the fact that smaller amounts of fluid can be used than with the Beckmann apparatus, which requires from 10 to 15 cc. of solution for a determination. Burian and Drucker[14] have reduced the size of the Beckmann apparatus so that determinations accurate to 0.005°C. can be made with but 2 cc. of fluid. Drucker and Schreiner[15] have been able to obtain the freezing-point lowering with but 0.005 cc. of fluid, with an accuracy of 0.01°C.

When only small amounts of sap can be obtained it has usually been the practice to add a measured volume of water to the crushed tissue and then press the thin mash, after thorough mixing. A correction for the dilution may be obtained by means of the percentage of water in the original tissue.

Ohlweiler[16] has put the thermo-electric method to another test, with gratifying results, and it appears that the electrical precision methods are to be generally recommended. Gortner and Harris[17] have made several suggestions, which have considerably reduced the time necessary to make a determination. By their procedure it is possible to make from twenty to twenty-five freezing-point determinations in a period of eight hours.

Bartley[18] has improved the old apparatus, so that the new form is much more convenient and almost automatic. The freezing tube, containing the fluid to be tested, is placed in another tube just a little larger, so that the two tubes are separated by a narrow space, which is filled with alcohol. These two tubes are then inserted into a Dewar tube which is about one-third full of ether or carbon bisulphide. By means of a Richards water aspirator, air is drawn through the ether, which serves as the freezing mixture. The whole system is clear and transparent, as there is no frosting of the Dewar tube. When a temperature of 0°C. is reached an electric stirrer is started and the fluid is allowed to under-cool. As soon as the temperature begins to rise the pump is stopped, and the reading is made when the mercury column stops rising.

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[18] Bartley, Arch. Diagnosis. 1913. (Cited in: Mathews, A. P., Physiological Chemistry. New York, 1915. Pages 201–202.)

The present paper presents a modification of the usual procedure for determining the osmotic concentration of plant saps by the freezing-point method. This modification consists simply in omitting the process of pressing, employing the crushed or ground tissue in the form of a pulpy mass. Such a simplification does not appear to decrease the value of the results obtained, and it does reduce the amount of labor and inconvenience. In this way all of the troubles arising from thick, gummy or mucilaginous fluids are completely avoided. As has been mentioned, both Müller Thurgau [1886] and Cavara [1905] made freezing-point determinations on the pulp of plant parts from which, on account of their gummy or viscid consistency, it was very difficult to express the juice. As far as the writers have been able to determine, no systematic study has heretofore been made to determine how satisfactory this method may be in general application.

The idea of obtaining cryoscopic determinations with the pulpy mass of ground plant tissue instead of with expressed sap was developed from a conversation with Dr G. J. Bouyoucos, concerning the use of the Beckmann thermometer for determining the concentration of the soil solution in the soil. This method is described by him in a recent publication.[19] Several months after the work here reported had been begun, the writer just mentioned published a note[20] suggesting that the method he had used for soils might be of some value in connection with plant pulps. The general conclusion stated in this note is that freezing-point depressions thus obtained may be expected to be greater than those obtained by other methods, but it will be seen that the studies to be reported below do not bear out this conclusion.

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[19] Bouyoucos, G. J., The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Exp. Sta. Tech. Bull. 24. 1916.

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ESSENTIALS OF THE FREEZING-POINT METHOD AS HERETOFORE EMPLOYED

Prior to 1913, plant saps were extracted from the tissue by merely crushing and pressing the latter, without any previous treatment. In that year, however, Dixon and Atkins [1913] observed that tissues that had been treated with chloroform before pressing yielded a sap of higher concentration than that obtained without such treatment. They also found the same thing to be true of tissues that had been desiccated and then made up to their original weight with water. They explain these differences by supposing that the impermeability, or very slow permeability, of the cell membranes, to some of the solutes of the vacuolar sap, held these back in the case of untreated tissues, so that these solutes were not nearly all obtained in the expressed fluid. After chloroform treatment or desiccation they supposed the permeability of the cell membranes to be very much greater. These authors also obtained higher concentrations of the expressed juice after treatment of the tissues with toluene vapor, and after freezing them, and they recommend the freezing method as the best. Liquid air was employed for the preliminary freezing. We agree, however, with Gortner and Harris [1914], that, although the use of liquid air is quick and very efficient, such extremely low temperatures are not actually required, and the cheap and easily obtained ice-salt mixture may be used instead. In our work we have had good success with carbon dioxide and ether, which are much less expensive and more easily procured, in most laboratories, than is liquid air. The method of preliminary treatment has been in quite general use since 1913. It does not, however, do away with all the difficulties encountered in getting a true sample of the plant sap.

THE MODIFIED METHOD HERE PROPOSED

The apparatus used in our tests was the Beckmann outfit ordinarily used for such work, and described in books on physical chemistry, consisting of a Beckmann thermometer, freezing tube, outer jacket, and a battery jar containing the freezing mixture. The freezing-point of distilled water was taken as zero and the lowering of the freezing-point of the pulp was obtained by substraction. When determining the freezing point of distilled water an electric stirring-device was used, consisting of battery, metronome, magnet, and platinum stirrer, but this was not employed in determinations made upon pulps. The pulp was allowed to under-cool about one degree, after which the beginning of solidification was brought about by rotating the thermometer backward and forward a few times in the pulp. When the under-cooled mass of pulp was thus disturbed the temperature began to rise almost immediately, and soon came to rest, after which the thermometer was tapped several times and the final reading then taken. This reading was considered as the freezing-point of the pulp tested. Correction for under-cooling has not been applied, since the under-cooling was always the same. Since, as has been especially emphasized by Shive,[21] the external air temperature exercises a marked influence on the apparent depressions of the freezing-point obtained by means of the Beckmann apparatus, the freezing of the pulp or expressed juice must always be carried out with approximately the same temperature of the surrounding air as prevailed during the determination of the freezing-point of distilled water, used for comparison with that of pulp or juice. The simplest way to avoid possible sources of error in this connection is to make a freezing-point determination on distilled water for each external air temperature at which pulps or juices are tested. Then the lowering for any test is considered as the difference between its freezing-point and that obtained on distilled water with the same room-temperature.

The property of the solution upon which its maximum possible osmotic pressure depends is approximately measured by its freezing-point lowering, and this property may be expressed in terms of pressure. Thus, according to the formula of Lewis,[22] $\Pi = 12.06\,\Delta - 0.021\,\Delta^2$, where Π is the maximum osmotic pressure, in atmospheres, at the freezing-point of the solutions, and Δ is the lowering of the freezing-point in centigrade degrees, below that of distilled water. With the aid of this formula Harris and Gortner[23] have prepared a table of the values of Π for the range, $\Delta = 0.001$°C. to $\Delta = 5.999$°C. This table has been employed in our deductions.

At the beginning of the work the material to be used was first ground and then frozen, but it was difficult to prevent some loss of sap in this way, and difficulty was also encountered in getting a perfect mixture of the material after thawing, since much of the sap had left the cells on grinding and had settled to the bottom of the mass. Consequently it was found better first to freeze the material and to grind it afterwards. In the earlier tests this preliminary freezing was carried out in large test tubes immersed in a mixture of salt and ice at a temperature of from −12° to −17°C. Sometimes, during cold weather, the material was placed out of doors over night for the preliminary freezing. In the remainder of the work it was frozen by carbon dioxide and ether. Carbon dioxide was obtained in the solid state by allowing the compressed gas to escape from the supply cylinder into a small cloth bag. The material to be frozen was placed in a beaker and completely covered with solid carbon dioxide. A small amount of ether was then added, until complete freezing had taken place. A temperature of approximately – 120°C. may be obtained in this way.

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[21] Shive, J. W., The freezing-points of Tottingham's nutrient solutions. Plant World **17**: 345–353. 1914.

[22] Lewis, G. N., The osmotic pressure of concentrated solutions and the laws of the perfect solution. Jour. Amer. Chem. Soc. **30**: 668–683. 1908.

[23] Harris, J. A. and R. A. Gortner, Notes on the calculation of the osmotic pressures of expressed vegetable saps from the depression of the freezing point, with a table for the values of Π for $\Delta = .001$°C. to $\Delta = 2.999$°C. Amer. Jour. Bot. **I**: 75–78. 1914.

Harris, J. A., An extension to 5.999°C. of tables to determine the osmotic pressures of expressed vegetable saps from the depression of the freezing point. Amer. Jour. Bot. **2**: 418–419. 1915.

The tissue is reduced to a finely divided condition by grating, or grinding in a food grinder. The ground material must be quickly and thoroughly mixed before sampling, since as would be expected, and as has indeed been found by other investigators, not all parts of a given organ give the same concentration of sap. Unless great care is taken in mixing, two or more samples of the same pulp do not have the same osmotic concentration.

Samples are placed in the freezing tubes and allowed to thaw completely, before the determination of the freezing-point is made. When the tissue is ground and pressed before the preliminary freezing many serious changes may take place; enzymes may be liberated, many new chemical reactions may be brought about and the solutions may change in various physical ways. After thawing, the material should be stirred with a glass or suitable wooden rod, to expel all air bubbles. Thawing may be completed within fifteen or twenty minutes at most, and the possibility of chemical change is thus very greatly reduced. When thawing is complete the thermometer is inserted, the tube is placed in the freezing mixture, and the material allowed to reach a temperature about 1° below its freezing-point. Solidification is then brought about, as has been stated, by turning the thermometer backward and forward a few times, to create a slight disturbance in the pulp. It has been found in practice that much more satisfactory results are obtained if the material is thus allowed to under-cool about 1°, than when solidification is brought about with less under-cooling. In the latter case the mercury rises to the freezing-point much more slowly and the determination of this point is consequently more difficult.

EXPERIMENTATION

The first problem that arose in planning this study was to determine whether it might be possible to obtain two or more results from different samples of the same material, that would agree within the range of experimental error. Adsorption is effective, no doubt, both with this modified method and when the sap is expressed, and it seems clear that whatever soluble material is adsorbed by the solid phases must be considered as inactive in affecting the lowering of the freezing-point as determined by either method.

We shall now present some of our experimental results. Table I gives the results obtained when two or more samples of the same material were tested by the pulp method, for potato tubers, leaves of cabbage heads, and apple fruits. The lowerings of the freezing-point (in degrees C.) and the derived osmotic concentrations for 0°C. (measured in atmospheres) are given.

From these data it appears that two determinations by the pulp method, from tissues supposed to be alike, may be expected to agree within much less than 1.0 per cent; the greatest variation recorded in table I is only 0.5 per cent.

TABLE I

Lowerings of the freezing-point and derived osmotic concentrations for duplicate tests with plant tissues, the ground pulp being used in all cases

TISSUE EMPLOYED	LOWERING OF FREEZING POINT (Δ)		PLUS OR MINUS VARIATION, FIRST TEST COMPARED TO SECOND		OSMOTIC CONCENTRATION DERIVED FROM Δ	
	First test	Second test	By difference	By ratio	First test	Second test
	deg. C.	*deg. C.*	*deg. C.*	*per cent*	*atm.*	*atm.*
Tubers of *Solanum tuberosum*	0.553	0.554	−0.001	−0.2	6.663	6.675
	0.817	0.813	+0.004	+0.5	9.839	9.791
	0.697	0.695	+0.002	+0.3	8.396	8.372
Storage leaves of *Brassica oleracea*	0.759	0.762	−0.003	−0.4	9.141	9.057
	0.806	0.803	+0.003	+0.4	9.707	9.671
Fruit of *Pyrus malus*	1.504	1.500	+0.004	+0.3	18.008	18.040
	1.487	1.485	+0.002	+0.1	17.884	17.860
	1.321	1.324	+0.003	+0.2	15.892	15.928

To determine whether or not freezing-point lowerings obtained by the pulp method might closely approximate those obtained by the usual method with expressed sap, the two methods were directly compared by a double series of tests. Of a given lot of pulp, one sample was pressed in a fruit-press and the resulting liquid was cleared by centrifuging for about 20 minutes, with a radius of about 15 cm. and a speed of rotation of 3000 per minute. The freezing-point lowering was then determined in the usual way. Another sample of the same pulp was directly tested, without pressing. The results of these parallel tests are given in table II.

It will be seen from table II that the difference between the freezing-point depression obtained for the pulp and that obtained for the expressed sap is less than 1.0 per cent, and probably easily within the range of experimental error. In some cases, however, this difference is much greater, being as great as 13.0 per cent in one case. This suggested that the first portions of juice expressed from a given mass of pulp might be of a different concentration from later portions derived from the same mass. If such were the case, the thoroughness of pressing might determine the concentration of the expressed sap, to a considerable degree. To test this suggestion, two lots of cabbage leaves (from the head) were frozen and ground to a pulp. In each case the resulting pulp was tested, after which it was partially pressed. Then this partly pressed pulp and the expressed juice were tested. Finally, the pulp was pressed again so as to obtain most of the juice still contained in it, and the somewhat dry residue, as well as the juice derived from the last pressing, were tested. The results are given in table III.

The values given in table III show, in one case, a pronounced lowering of the concentration of the expressed sap from the second pressing, and, in the other case, a slight increase. In both cases the freezing-point depression of the pulp decreased in magnitude progressively, as the juice was pressed out. The concentration is dependent on the thoroughness of the pressing.

TABLE II

Comparisons between freezing-point lowerings of triturated plant tissues and of the juice expressed therefrom

SOURCE OF MATERIAL	TEST NUMBER	FREEZING-POINT DEPRESSION (Δ) For pulp	For expressed sap	Plus or minus variation, pulp test compared to sap test: By difference	By ratio
		deg. C.	*deg. C.*	*deg. C.*	*per cent*
Tubers of *Solanum tuberosum*	1	0.625	0.553	+0.072	+13.02
	2	0.635	0.645	−0.010	− 1.55
	3	0.687	0.686	+0.001	+ 0.15
	4	0.485	0.485	....	....
	5	0.700	0.697	+0.003	+ 0.43
	6	0.822	0.817	+0.005	+ 0.61
	7	0.733	0.730	+0.003	+ 0.41
	8	0.778	0.791	−0.013	− 1.64
	9	0.931	0.936	−0.005	− 0.53
Fruit of *Citrus sinensis*	1	1.511	1.515	−0.004	− 0.26
	2	1.521	1.526	−0.005	− 0.33
Fruit of *Pyrus malus*	1	1.504	1.498	+0.006	+ 0.40
	2	1.487	1.482	+0.005	+ 0.34
	3	1.321	1.319	+0.002	+ 0.15
Storage leaves of *Brassica oleracea*	1	0.759	0.754	+0.005	+ 0.66
	2	1.099	1.098	+0.001	+ 0.09
	3	0.815	0.806	+0.009	+ 1.12
Fruit of *Citrus limonia*		1.212	1.215	−0.003	− 0.25
Fruit of *Citrus grandis*		1.171	1.168	+0.003	+ 0.26

TABLE III

Freezing-point lowerings of unpressed, partly pressed and rather thoroughly pressed pulp of storage leaves of Brassica oleracea, together with similar data for the expressed juice derived from the first and from the second extraction

EXPERIMENT NUMBER	FREEZING-POINT DEPRESSION (Δ) Pulp: Original, unpressed	Partly pressed	Thoroughly pressed	Expressed juice: First pressing	Second pressing
	deg. C.	*deg. C.*	*deg. C.*	*deg. C.*	*deg. C.*
1	0.819	0.521	0.391	1.223	0.727
2	0.887	0.858	0.350	0.858	0.908

A comparison of the concentration values obtained for unpressed pulp and for the juice obtained from a very thorough pressing, was carried out for potato tubers, cabbage leaves, apple fruits and onion bulbs. In this case carbon dioxide and ether were used for the preliminary freezing and a hydraulic press giving a pressure of 350 kilograms per square centimeter was employed. The ground material was divided into two portions; one part was tested without pressing and the remainder was pressed, and the expressed juice was tested. The results are set forth in table IV.

The results recorded in table IV indicate very clearly that the osmotic concentration of sap very thoroughly expressed closely approaches that of the unpressed pulp. The greatest difference recorded is only 0.4 per cent. From this it appears that the results obtained by using the unpressed pulp may be considered as in practical agreement with those obtained with expressed sap, when the pressing has been thoroughly carried out.

TABLE IV

Freezing-point lowerings for pulp and for very thoroughly expressed sap from four different plant tissues

SOURCE OF MATERIAL	TEST NUMBER	DEPRESSION OF FREEZING-POINT (Δ)			
		Unpressed pulp	Expressed sap	Difference, pulp compared to sap	Ratio, sap to pulp
		deg. C.	*deg. C.*	*deg. C.*	
Tubers of *Solanum tuberosum*	1	0.837	0.840	−0.003	1.004
	2	0.696	0.698	−0.002	1.003
Fruits of *Pyrus malus*	1	1.503	1.505	−0.002	1.001
	2	1.531	1.525	+0.006	0.996
Storage leaves of *Brassica oleracea*	1	0.943	0.939	+0.004	0.996
	2	0.961	0.957	+0.004	0.996
Bulbs of *Allium cepa*	1	1.171	1.169	+0.002	0.998
	2	1.174	1.174	......	1.000

All of the material so far dealt with has been rather watery, and it was thought desirable to test the method here considered in cases where the moisture content of the tissues was not so great. For this purpose the tops and roots of young maize plants were used separately. The plants had been grown 7 weeks in 12-inch pots of garden loam, in the green-house. Six different cultures were employed, each one having a different mean moisture content of the soil for the period of growth, so as to make a preliminary test of the relation of soil moisture content to the osmotic concentration of the plant pulp.

The soil moisture of these cultures was controlled by means of the Livingston porous cup auto-irrigator.[24] The porous cups, of the type ordinarily used for cylindrical atmometers, were embedded in the soil and connected by lead tubing to receptacles of distilled water below. The height of the pot above the water reservoir was arranged so that the six pots were maintained with different soil moisture contents, the content of each pot being held nearly uniform throughout the growth of the plants.

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[24] Livingston, B. E., A method for controlling plant moisture. Plant World **11**: 39–40. 1908.
Hawkins, Lon A., The porous clay cup for the automatic watering of plants. Plant World **13**: 220–227. 1910.
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Livingston, B. E. and Lon A. Hawkins, The water relation between plant and soil. Carnegie Inst. Wash. Pub. **204**: 5–48. 1915.

When the plants were harvested, the roots were thoroughly washed out in running tap water, separated from the aerial portions of the plant, dried with cloth and then with filter-paper, and then frozen with CO_2 and ether. They were finally ground before placing in the freezing tube to thaw. The tops (stems and leaves) were cut up coarsely and frozen in the same way as the roots. Afterwards they were ground and placed in the freezing-tube to thaw before the thermometer was inserted and the freezing-point determinations were made. Table V shows the results obtained.

TABLE V

Comparative freezing-point lowerings of ground tops and ground roots of maize plants grown with different degrees of soil moisture

CULTURE NUMBER	SOIL MOISTURE CONTENT, ON DRY WEIGHT BASIS	FREEZING-POINT DEPRESSION (Δ)	
		Tops	Roots
	per cent	*deg. C.*	*deg. C.*
1	31	1.835	0.492
2	23	1.920	0.600
3	16	2.027	0.647
4	14	2.084	0.767
5	13	2.120	0.942
6	11	2.204	0.995

It will be seen from table V that the freezing-point lowering was always much less for roots than for tops. It will also be observed that, as the water-content of the soil decreases, there is an increase in the depression of the freezing-point for both roots and tops.[25]

Owing to lack of material, only one comparison was made between unpressed pulp and the sap expressed therefrom, and both results were the same in this case; both pulps and juice gave a freezing-point lowering of 1.835°C. The plants were from culture 1, and both roots and tops were combined in the pulp here used.

These results indicate not only that the method employing tissue pulps is applicable in the case of somewhat dry material, but they also bring out what appear to be an important relation between soil-moisture condition and the freezing-point lowering of the plant pulp. The weight-percentage values for soil-moisture content in these cultures may be taken as approximately proportional to the power of the soil to supply moisture to the plants, since all the soils were the same and since the packing in the pots was very nearly uniform. This relation between the freezing-point lowerings for pulps from tops and from roots, and for plants grown under different conditions of soil moisture, deserves through-going attention, for it must be a very important relation in the growth of plants in the field.

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[25] In regard to differences between freezing-point depressions of roots and stems: Dixon, H. H., and W. R. G. Atkins, Variations in the osmotic pressure of the sap of Ilex Aquifolium. Sci. Proc. Roy. Dublin Soc. N. S. **13**: 229-238. 1912. Pages 235 and 238. Idem, Osmotic pressure in plants. I. Methods of extracting sap from plant organs. Ibid. **13**: 422-433. 1913. Page 427, tabulation. Idem, Osmotic pressure in plants. II. Cryoscopic and conductivity measurements on some vegetable saps. Ibid. **13**: 434-440. 1913. Pages 436, tabulation, and 438. Idem, On osmotic pressure in plants; and on a thermo-electric method of determining freezing-points. Ibid. **12**: 275-311. 1910. Pages 309 and 310.

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